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PCT

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>5</sup> :</b>  <b>A61K 37/02, G01N 33/68</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 94/03200</b>  <b>(43) International Publication Date:</b> <b>17 February 1994 (17.02.94)</b>									
<b>(21) International Application Number:</b> <b>PCT/US93/07231</b> <b>(22) International Filing Date:</b> <b>29 July 1993 (29.07.93)</b>  <b>(30) Priority data:</b> <table border="0"> <tr> <td>922,813</td> <td>31 July 1992 (31.07.92)</td> <td>US</td> </tr> <tr> <td>029,335</td> <td>4 March 1993 (04.03.93)</td> <td>US</td> </tr> <tr> <td>040,510</td> <td>31 March 1993 (31.03.93)</td> <td>US</td> </tr> </table> <b>(71) Applicant:</b> <b>CREATIVE BIOMOLECULES, INC. [US/US]; 45 South Street, Hopkinton, MA 01748 (US).</b>  <b>(72) Inventors:</b> <b>RUEGER, David, C. ; 19 Downey Street, Hopkinton, MA 01748 (US). KUBERASAMPATH, Thanagavel ; Six Spring Street, Medway, MA 02053 (US). OPPERMAN, Hermann ; 25 Summer Hill Road, Medway, MA 02053 (US). OZKAYNAK, Engin ; 44 Purdue Drive, Milford, MA 01757 (US). PANG, Roy, H., L. ; 15 Partridge Road, Etna, NH 03750 (US). COHEN, Charles, M. ; 98 Winthrop Street, Medway, MA 02053 (US).</b>		922,813	31 July 1992 (31.07.92)	US	029,335	4 March 1993 (04.03.93)	US	040,510	31 March 1993 (31.03.93)	US	<b>(74) Agent:</b> <b>KELLEY, Robin, D.; Testa, Hurwitz &amp; Thibault, Exchange Place, 53 State Street, Boston, MA 02109 (US).</b>  <b>(81) Designated States:</b> <b>AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b>  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
922,813	31 July 1992 (31.07.92)	US									
029,335	4 March 1993 (04.03.93)	US									
040,510	31 March 1993 (31.03.93)	US									

**(54) Title:** **MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR****(57) Abstract**

Disclosed are therapeutic treatment methods, compositions and devices for maintaining neural pathways in a mammal, including enhancing survival of neurons at risk of dying, inducing cellular repair of damaged neurons and neural pathways, and stimulating neurons to maintain their differentiated phenotype. In one embodiment, the invention provides means for stimulating CAM expression in neurons. The invention also provides means for evaluating the status of nerve tissue, including means for detecting and monitoring neuropathies in a mammal. The methods, devices and compositions include a morphogen-stimulating agent provided to the mammal in a therapeutically effective concentration.

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Morphogen-Induced Nerve Regeneration and Repair

5

BACKGROUND OF THE INVENTION

The present invention relates to methods for enhancing the survival of neuronal cells in vivo and to  
10 methods, compositions and devices for maintaining neural pathways in vivo. More particularly, the invention provides methods for enhancing survival of neuronal cells at risk of dying, including methods for redifferentiating transformed cells of neural origin  
15 and methods for maintaining phenotypic expression of differentiated neuronal cells. The invention also provides means for repairing damaged neural pathways, including methods for stimulating axonal growth over extended distances, and methods for alleviating  
20 immunologically-related nerve tissue damage. In a particular embodiment of the invention, this invention provides a method for stimulating cell adhesion molecule expression in cells, and particularly nerve cell adhesion molecule expression in neurons. Finally,  
25 the invention provides means for evaluating nerve tissue stasis and identifying neural dysfunction in a mammal.

The mammalian nervous system comprises a peripheral  
30 nervous system (PNS) and a central nervous system (CNS, comprising the brain and spinal cord), and is composed of two principal classes of cells: neurons and glial cells. The glial cells fill the spaces between neurons, nourishing them and modulating their function.  
35 Certain glial cells, such as Schwann cells in the PNS

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and oligodendrocytes in the CNS, also provide a protective myelin sheath that surrounds and protects neuronal axons, which are the processes that extend from the neuron cell body and through which the electric impulses of the neuron are transported. In the peripheral nervous system, the long axons of multiple neurons are bundled together to form a nerve or nerve fiber. These, in turn, may be combined into fascicles, wherein the nerve fibers form bundles embedded, together with the intraneural vascular supply, in a loose collagenous matrix bounded by a protective multilamellar sheath. In the central nervous system, the neuron cell bodies are visually distinguishable from their myelin-ensheathed processes, and are referenced in the art as grey and white matter, respectively.

During development, differentiating neurons from the central and peripheral nervous systems send out axons that must grow and make contact with specific target cells. In some cases, growing axons must cover enormous distances; some grow into the periphery, whereas others stay confined within the central nervous system. In mammals, this stage of neurogenesis is complete during the embryonic phase of life and neuronal cells do not multiply once they have fully differentiated.

Accordingly, the neural pathways of a mammal are particularly at risk if neurons are subjected to mechanical or chemical trauma or to neuropathic degeneration sufficient to put the neurons that define the pathway at risk of dying. A host of neuropathies, some of which affect only a subpopulation or a system of neurons in the peripheral or central nervous systems

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have been identified to date. The neuropathies, which may affect the neurons themselves or the associated glial cells, may result from cellular metabolic dysfunction, infection, exposure to toxic agents, autoimmunity dysfunction, malnutrition or ischemia. In some cases the cellular dysfunction is thought to induce cell death directly. In other cases, the neuropathy may induce sufficient tissue necrosis to stimulate the body's immune/inflammatory system and the mechanisms of the body's immune response to the initial neural injury then destroys the neurons and the pathway defined by these neurons.

Currently no satisfactory method exists to repair the damage caused by these neuropathies, which include multiple sclerosis, amyotrophic lateral sclerosis (ALS), Huntington's chorea, Alzheimer's disease, Parkinson's disease (parkinsonism), and metabolically derived disorders, such as hepatic encephalopathy. Current attempts to counteract the effects of severe traumatic or neural degenerative lesions of the brain and/or spinal cord have to date primarily involved implantation of embryonic neurons in an effort to replace functionally, or otherwise compensate for, lost or deficient neurons. Currently, however, human fetal cell transplantation research is severely restricted. Administration of neurotrophic factors such as nerve growth factor and insulin-like growth factor also have been suggested to stimulate neuronal growth within the CNS. (See, for example, Lundborg, (1987) Acta Orthop. Scand. 58:145-169 and US Pat. No. 5,093,317.) Administration of neurotrophic factors to the CNS requires bypassing the blood-brain barrier. The barrier may be overcome by direct infusion, or by modifying the molecule to enhance its transport across

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the barrier, as by chemical modification or conjugation, or by molecule truncation. Schwann cells also have been grafted to a site of a CNS lesion in an attempt to stimulate and maintain growth of damaged neuronal processes (Paino et al. (1991) Exp. Neurology 114(2):254-257).

Where the damaged neural pathway results from CNS axonal damage, autologous peripheral nerve grafts have been used to bridge lesions in the central nervous system and to allow axons to make it back to their normal target area. In contrast to CNS neurons, neurons of the peripheral nervous system can extend new peripheral processes in response to axonal damage. This regenerative property of peripheral nervous system axons is thought to be sufficient to allow grafting of these segments to CNS axons. Successful grafting appears to be limited, however, by a number of factors, including the length of the CNS axonal lesion to be bypassed, and the distance of the graft sites from the CNS neuronal cell bodies, with successful grafts occurring near the cell body.

Within the peripheral nervous system, this cellular regenerative property of neurons has limited ability to repair function to a damaged neural pathway. Specifically, the new axons extend randomly, and are often misdirected, making contact with inappropriate targets that can cause abnormal function. For example, if a motor nerve is damaged, regrowing axons may contact the wrong muscles, resulting in paralysis. In addition, where severed nerve processes result in a gap of longer than a few millimeters, e.g., greater than 10

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- millimeters (mm), appropriate nerve regeneration does not occur, either because the processes fail to grow the necessary distance, or because of misdirected axonal growth. Efforts to repair peripheral nerve damage by surgical means has met with mixed results, particularly where damage extends over a significant distance. In some cases, the suturing steps used to obtain proper alignment of severed nerve ends stimulates the formulation of scar tissue which is thought to inhibit axon regeneration. Even where scar tissue formation has been reduced, as with the use of nerve guidance channels or other tubular prostheses, successful regeneration generally still is limited to nerve damage of less than 10 millimeters in distance.
- In addition, the reparative ability of peripheral neurons is significantly inhibited where an injury or neuropathy affects the cell body itself or results in extensive degeneration of a distal axon.
- Mammalian neural pathways also are at risk due to damage caused by neoplastic lesions. Neoplasias of both the neurons and glial cells have been identified. Transformed cells of neural origin generally lose their ability to behave as normal differentiated cells and can destroy neural pathways by loss of function. In addition, the proliferating tumors may induce lesions by distorting normal nerve tissue structure, inhibiting pathways by compressing nerves, inhibiting cerebrospinal fluid or blood supply flow, and/or by stimulating the body's immune response. Metastatic tumors, which are a significant cause of neoplastic lesions in the brain and spinal cord, also similarly may damage neural pathways and induce neuronal cell death.



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One type of morphoregulatory molecule associated with neuronal cell growth, differentiation and development is the cell adhesion molecule ("CAM"), most notably the nerve cell adhesion molecule (N-CAM). CAMs belong to the immunoglobulin super-family and mediate cell-cell interactions in developing and adult tissues through homophilic binding, i.e., CAM-CAM binding on apposing cells. A number of different CAMs currently have been identified. Of these, the most thoroughly studied to date are N-CAM and L-CAM (liver cell adhesion molecules), both of which have been identified on all cells at early stages of development, as well as in different adult tissues. In neural tissue development, N-CAM expression is believed to be important in tissue organization, neuronal migration, nerve-muscle tissue adhesion, retinal formation, synaptogenesis, and neural degeneration. Reduced N-CAM expression also is thought to be associated with nerve dysfunction. For example, expression of at least one form of N-CAM, N-CAM-180, is reduced in a mouse dysmyelinating mutant (Bhat (1988) Brain Res. 452:373-377). Reduced levels of N-CAM also have been associated with normal pressure hydrocephalus (Werdelin (1989) Acta Neurol. Scand. 79:177-181), and with type II schizophrenia (Lyons et al., (1988) Biol. Psychiatry 23:769-775.) In addition, antibodies to N-CAM have been shown to disrupt functional recovery in injured nerves (Remsen (1990) Exp. Neurobiol. 110:268-273).

It is an object of this invention to provide methods for enhancing survival of neurons at risk of dying in a mammal. Another object is to provide methods for maintaining neural pathways in vivo at risk of injury, or following damage to nerve tissue due to mechanical or chemical trauma, a neuropathy, or a

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neoplastic lesion. Another object is to provide compositions and devices for repairing gaps in a neural pathway of the peripheral nervous system. Yet another object is to provide a means for redifferentiating transformed cells defining neural pathways, particularly transformed cells of neural origin. Another object is to provide a means for stimulating CAM expression, particularly N-CAM expression in a cell. Yet another object is to provide methods for monitoring the status of nerve tissue by monitoring fluctuations in protein levels present in nerve tissue, serum and/or cerebrospinal fluid. These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

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Summary of the Invention

The present invention provides methods and  
5 compositions for maintaining neural pathways in a  
mammal in vivo, including methods for enhancing the  
survival of neural cells.

In one aspect, the invention features compositions  
10 and therapeutic treatment methods that comprise the  
step of administering to a mammal a therapeutically  
effective amount of a morphogenic protein  
("morphogen"), as defined herein, upon injury to a  
neural pathway, or in anticipation of such injury, for  
15 a time and at a concentration sufficient to maintain  
the neural pathway, including repairing damaged  
pathways, or inhibiting additional damage thereto.

In another aspect, the invention features  
20 compositions and therapeutic treatment methods for  
maintaining neural pathways in a mammal in vivo which  
include administering to the mammal, upon injury to a  
neural pathway or in anticipation of such injury, a  
compound that stimulates in vivo a therapeutically  
25 effective concentration of an endogenous morphogen  
within the body of the mammal sufficient to maintain  
the neural pathway, including repairing damaged  
pathways or inhibiting additional damage thereto.  
These compounds are referred to herein as morphogen-  
30 stimulating agents, and are understood to include  
substances which, when administered to a mammal, act on  
tissue(s) or organ(s) that normally are responsible

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for, or capable of, producing a morphogen and/or secreting a morphogen, and which cause the endogenous level of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or  
5 secretion of an endogenous morphogen.

In particular, the invention provides methods for enhancing the survival of neurons at risk of dying, including protecting neurons from the tissue  
10 destructive effects associated with the body's immune/inflammatory response to a nerve injury. The invention also provides methods for stimulating neurons to maintain their differentiated phenotype, including inducing the redifferentiation of transformed cells of  
15 neuronal origin to a morphology characteristic of untransformed neurons. In one embodiment, the invention provides means for stimulating production of cell adhesion molecules in cells, particularly nerve cell adhesion molecules (N-CAM) in neurons. The  
20 invention also provides methods, compositions and devices for stimulating cellular repair of damaged neurons and neural pathways, including regenerating damaged axons of the peripheral and central nervous systems. In addition, the invention also provides  
25 means for evaluating the status of nerve tissue, and for detecting and monitoring neuropathies in a mammal by monitoring fluctuations in the morphogen levels or endogenous morphogen antibody levels present in a mammal's serum or cerebrospinal fluid.

30

As used herein, a "neural pathway" describes a nerve circuit for the passage of electric signals from a source to a target cell site. The pathway includes the neurons through which the electric impulse is.

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transported, including groups of interconnecting neurons, the nerve fibers formed by bundled neuronal axons, and the glial cells surrounding and associated with the neurons.

5

In one aspect of the invention, the morphogens described herein are useful in repairing damaged neural pathways of the peripheral nervous system. In particular, the morphogens are useful for repairing  
10 damaged pathways, including transected or otherwise damaged nerve fibers (nerves) requiring regeneration of neuronal processes, particularly axons, over extended distances to bridge a gap in the nerve itself, or between the nerve and a post-synaptic cell.

15 Specifically, the morphogens described herein are capable of stimulating complete axonal nerve regeneration, including vascularization and reformation of the protective myelin sheath. The morphogen preferably is provided to the site of injury dispersed  
20 in a biocompatible, bioresorbable carrier material capable of maintaining the morphogen at the site and, where necessary, means for directing axonal growth from the proximal to the distal ends of a severed neuron or nerve. For example, means for directing axonal growth  
25 may be required where nerve regeneration is to be induced over an extended distance, such as greater than 10 mm. Many carriers capable of providing these functions are envisioned. For example, useful carriers include substantially insoluble materials or viscous  
30 solutions prepared as disclosed herein comprising laminin, hyaluronic acid or collagen, or other suitable synthetic, biocompatible polymeric materials such as polylactic, polyglycolic or polybutyric acids and/or copolymers thereof. The currently preferred carrier  
35 comprises an extracellular matrix composition, such as

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one described herein derived, for example, from mouse sarcoma cells. Also envisioned as especially useful are brain tissue-derived extracellular matrices.

5        In a particularly preferred embodiment, the morphogen is provided to the site as part of a device wherein the morphogen is disposed in a nerve guidance channel which spans the distance of the damaged pathway. The channel acts both as a protective  
10        covering and a physical means for guiding growth of a neuronal process such as an axon. Useful channels comprise a biocompatible membrane or casing, which may be tubular in structure, having a dimension sufficient to span the gap or break in the nerve to be repaired,  
15        and having openings adapted to receive severed nerve ends. The casing or membrane may be made of any biocompatible, nonirritating material, such as silicone or a biocompatible polymer such as polyethylene or polyethylene vinyl acetate. The casing also may be  
20        composed of biocompatible, bioresorbable polymers, including, for example, collagen, hyaluronic acid, polylactic, polybutyric and polyglycolic acids. In a currently preferred embodiment, the outer surface of the channel is substantially impermeable.

25

      The morphogen may be disposed in the channel in association with a biocompatible carrier material, or it may be adsorbed to or otherwise associated with the inner surface of the casing, such as is described in  
30        U.S. Pat. No. 5,011,486, provided that the morphogen is accessible to the severed nerve ends. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative  
35        shapes may be employed. The lumen of the guidance

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channels may, for example, be oval or even square in cross section. Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may  
5 be secured to the nerve guidance channels by means of sutures, biocompatible adhesives such as fibrin glue, or other means known in the medical art.

The morphogens described herein also are envisioned  
10 to be useful in autologous peripheral nerve segment implants to bypass damaged neural pathways in the central nervous system, such as in the repair of damaged or detached retinas, or other damage to the optic nerve. Here the morphogen is provided to the  
15 site of attachment to stimulate axonal growth at the graft site, particularly where the damaged axonal segment to be bypassed occurs far from the neuronal cell body.

20 The morphogens described herein also are useful for enhancing survival of neuronal cells at risk of dying, thereby preventing, limiting or otherwise inhibiting damage to neural pathways. Non-mitotic neurons are at risk of dying as a result of a neuropathy or other  
25 cellular dysfunction of a neuron or glial cell inducing cell death, or following a chemical or mechanical lesion to the cell or its surrounding tissue. The chemical lesions may result from known toxic agents, including lead, ethanol, ammonia, formaldehyde and many  
30 other organic solvents, as well as the toxins in cigarette smoke and opiates. Excitatory amino acids, such as glutamate also may play a role in the pathogenesis of neuronal cell death (see Freese et al. (1990) Brain Res. 521:254-264). Neuronal cell death  
35 also is thought to be a significant contributing factor

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in a number of neurodegenerative diseases, including Alzheimer's disease, Huntington's chorea, and Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. The etiology of these neuropathies  
5 may be metabolic, as results in hepatic encephalopathy, infectious, toxic, autoimmune, nutritional or ischemic. In addition, ethanol and a number of other toxins also have been identified as significant contributing factors in neurodegenerative diseases. The morphogens  
10 described herein may be provided to cells at risk of dying to enhance their survival and thereby protect the integrity of the neural pathway. The morphogens may be provided directly to the site, or they may be provided systemically. Alternatively, as described above, an  
15 agent capable of stimulating endogenous morphogen expression and/or secretion, preferably in cells associated with the nerve tissue of interest, may be administered to the mammal.

20 In another aspect of the invention, the method disclosed is useful for redifferentiating transformed cells, particularly transformed cells of neuronal or glial origin, such that the morphogen-treated cells are induced to display a morphology characteristic of  
25 untransformed cells. Where the transformed cells are cells of neuronal origin, morphogen treatment preferably induces cell rounding and cell aggregation (clumping), cell-cell adhesion, neurite outgrowth formation and elongation, and N-CAM production. The  
30 methods described herein are anticipated to substantially inhibit or reduce neural cell tumor formation and/or proliferation in nerve tissue. It is anticipated that the methods of this invention will be useful in substantially reducing the effects of various  
35 carcinomas of nerve tissue origin such as



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retinoblastomas, neuroblastomas, and gliomas or glioblastomas. In addition, the method also is anticipated to aid in inhibiting neoplastic lesions caused by metastatic tissue. Metastatic tumors are one  
5 of the most common neoplasms of the CNS, as they can reach the intracranial compartment through the bloodstream. Metastatic tumors may damage neural pathways for example, by distorting normal nerve tissue structure, compressing nerves, blocking flow of  
10 cerebrospinal fluid or the blood supply nourishing brain tissue, and/or by stimulating the body's immune response.

In another aspect of the invention, the morphogens  
15 described herein are useful for providing neuroprotective effects to alleviate neural pathway damage associated with the body's immune/inflammatory response to an initial injury to nerve tissue. Such a response may follow trauma to nerve tissue, caused, for  
20 example, by an autoimmune dysfunction, neoplastic lesion, infection, chemical or mechanical trauma, disease, by interruption of blood flow to the neurons or glial cells, for example following ischemia or hypoxia, or by other trauma to the nerve or surrounding  
25 material. For example, the primary damage resulting from hypoxia or ischemia-reperfusion following occlusion of a neural blood supply, as in an embolic stroke, is believed to be immunologically associated. In addition, at least part of the damage associated  
30 with a number of primary brain tumors also appears to be immunologically related. Application of the morphogen directly to the cells to be treated, or providing the morphogen to the mammal systemically, for example, intravenously or indirectly by oral  
35 administration, may be used to alleviate and/or inhibit

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the immunologically related response to a neural injury. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury, also may be used. Where the injury is to be induced, as during surgery or other aggressive clinical treatment, the morphogen or agent may be provided prior to induction of the injury to provide a neuroprotective effect to the nerve tissue at risk.

10

In still another aspect, the invention described herein provides methods for supporting the growth and maintenance of differentiated neurons, including inducing neurons to continue expressing their phenotype. It is anticipated that this activity will be particularly useful in the treatment of nerve tissue disorders where loss of function is caused by reduced or lost cellular metabolic function and cells become senescent or quiescent, such as is thought to occur in aging cells and to be manifested in Alzheimer's disease. Application of the morphogen directly to cells to be treated, or providing it systemically by parenteral or oral administration stimulates these cells to continue expressing their phenotype, significantly inhibiting and/or reversing the effects of the cellular metabolic dysfunction, thereby maintaining the neural pathway at risk. Alternatively, administration of an agent capable of stimulating endogenous morphogen expression and/or secretion in vivo may be used.

30

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In still another aspect, the invention provides methods for stimulating CAM expression levels in a cell, particularly N-CAM expression in neurons. CAMs are molecules defined as carrying out cell-cell interactions necessary for tissue formation. CAMs are believed to play a fundamental regulatory role in tissue development, including tissue boundary formation, embryonic induction and migration, and tissue stabilization and regeneration. Altered CAM levels have been implicated in a number of tissue disorders, including congenital defects, neoplasias, and degenerative diseases.

In particular, N-CAM expression is associated with normal neuronal cell development and differentiation, including retinal formation, synaptogenesis, and nerve-muscle tissue adhesion. Inhibition of one or more of the N-CAM isoforms is known to prevent proper tissue development. Altered N-CAM expression levels also are associated with neoplasias, including neuroblastomas (see *infra*), as well as with a number of neuropathies, including normal pressure hydrocephalous and type II schizophrenia. Application of the morphogen directly to the cells to be treated, or providing the morphogen to the mammal systemically, for example, parenterally, or indirectly by oral administration, may be used to induce cellular expression of one or more CAMs, particularly N-CAMs. Alternatively, administration of an agent capable of stimulating morphogen expression and/or secretion in vivo, preferably at the site of injury, also may be used to induce CAM production.

CAMs also have been postulated as part of a morphoregulatory pathway whose activity is induced by a to date unidentified molecule (See, for example,

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Edelman, G.M. (1986) Ann. Rev. Cell Biol. 2:81-116). Without being limited to any given theory, the morphogens described herein may act as the inducer of this pathway.

5

Finally, modulations of endogenous morphogen levels may be monitored as part of a method of detecting nerve tissue dysfunction. Specifically, modulations in endogenous morphogen levels are anticipated to reflect changes in nerve tissue status. Morphogen expression may be monitored directly in biopsied cell samples, in cerebrospinal fluid, or serum. Alternatively, morphogen levels may be assessed by detecting changes in the levels of endogenous antibodies to the morphogen. For example, one may obtain serum samples from a mammal, and then detect the concentration of morphogen or antibody present in the fluid by standard protein detection means known to those skilled in the art. As an example, binding protein capable of interacting specifically with the morphogen of interest such as an anti-morphogen antibody may be used to detect a morphogen in a standard immunoassay. The morphogen levels detected then may be compared to a previously determined standard or reference level, with changes in the detected levels being indicative of the status of the tissue.

In one preferred embodiment of the invention, the morphogen or morphogen-stimulating agent is administered systemically to the individual, e.g., orally or parenterally. In another embodiment of the invention, the morphogen may be provided directly to the nerve tissue, e.g., by injection to the cerebral spinal fluid or to a nerve tissue locus.

35

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In any treatment method of the invention, "administration of morphogen" refers to the administration of the morphogen, either alone or in combination with other molecules. For example, the  
5 mature form of the morphogen may be provided in association with its precursor "pro" domain, which is known to enhance the solubility of the protein. Other useful molecules known to enhance protein solubility include casein and other milk components, as well as  
10 various serum proteins. Additional useful molecules which may be associated with the morphogen or morphogen-stimulating agent include tissue targeting molecules capable of directing the morphogen or morphogen-stimulating agent to nerve tissue. Tissue  
15 targeting molecules envisioned to be useful in the treatment protocols of this invention include antibodies, antibody fragments or other binding proteins which interact specifically with surface molecules on nerve tissue cells.

20

Still another useful tissue targeting molecule is part or all of the morphogen precursor "pro" domain, particularly that of OP-1 or GDF-1. These proteins are found naturally associated with nerve tissue but also  
25 may be synthesized in other tissues and targeted to nerve tissue after secretion from the synthesizing tissue. For example, while the protein has been shown to be active in bone tissue, the primary source of OP-1 synthesis appears to be the tissue of the urogenic  
30 system (e.g., renal and bladder tissue), with secondary expression levels occurring in the brain, heart and lungs (see below.) Moreover, the protein has been identified in serum, saliva and various milk forms. In addition, the secreted form of the protein comprises  
35 the mature dimer in association with the pro domain of

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the intact morphogen sequence. Accordingly, the associated morphogen pro domains may act to target specific morphogens to different tissues in vivo.

5 Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of  
10 bone remodeling sites.

Finally, the morphogens or morphogen-stimulating agents provided herein also may be administered in combination with other molecules known to be beneficial  
15 in maintaining neural pathways, including, for example, nerve growth factors and anti-inflammatory agents.

Where the morphogen is intended for use as a therapeutic for disorders of the CNS, an additional  
20 problem must be addressed: overcoming the so-called "blood-brain barrier", the brain capillary wall structure that effectively screens out all but selected categories of molecules present in the blood, preventing their passage into the brain. The  
25 blood-brain barrier may be bypassed effectively by direct infusion of the morphogen or morphogen-stimulating agent into the brain. Alternatively, the morphogen or morphogen-stimulating agent may be modified to enhance its transport across the  
30 blood-brain barrier. For example, truncated forms of the morphogen or a morphogen-stimulating agent may be most successful. Alternatively, the morphogen or

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morphogen-stimulating agent may be modified to render it more lipophilic, or it may be conjugated to another molecule which is naturally transported across the barrier, using standard means known to those skilled in the art, as, for example, described in Pardridge, Endocrine Reviews 7:314-330 (1986) and U.S. Pat. No. 4,801,575.

Accordingly, as used herein, a functional "analog" of a morphogen refers to a protein having morphogenic biological activity but possessing additional structural differences compared to a morphogen as defined herein, e.g., having additional chemical moieties not normally a part of a morphogen. Such moieties (introduced, for example, by acylation, alkylation, cationization, or glycosylation reactions, or other means for conjugating the moiety to the morphogen) may improve the molecule's solubility, absorption, biological half-life, or transport, e.g., across the blood-brain barrier.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos. 5-14), and the recently identified 60A protein (from *Drosophila*, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- $\beta$  super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a

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precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

15

TABLE I

20	"OP-1"	Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature,
25		
30		
35		



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morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

- 5 "OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)
- 30 "CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA

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("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

"DPP(fx)"

refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

25 "Vgl(fx)"

refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

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- 5 "Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.
- 10
- 15 "GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32. The prodomain likely extends from the signal peptide clavage site to residue 214; the mature protein likely is defined by residues 215-372.
- 20
- 25 "60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.)
- 30

- 25 -

The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.

5

"BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.

10

15

"BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

20

25

"BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appear sin Celeste, et al.

30

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(1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

5

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of

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acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer,  $\alpha$ -amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)  
1 5

Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31), listed below. These



- 29 -

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa  
 25 30  
 Xaa Pro Xaa Xaa Xaa Xaa Xaa  
 35  
 5 Xaa Xaa Xaa Asn His Ala Xaa Xaa  
 40 45  
 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa  
 50  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys  
 10 55 60  
 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa  
 65  
 Xaa Xaa Xaa Leu Xaa Xaa Xaa  
 70 75  
 15 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa  
 80  
 Xaa Xaa Xaa Xaa Met Xaa Val Xaa  
 85 90  
 Xaa Cys Gly Cys Xaa  
 20 95

wherein each Xaa is independently selected from a group  
 of one or more specified amino acids defined as  
 follows: "Res." means "residue" and Xaa at res.4 =  
 (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or  
 25 Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu  
 or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn);  
 Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile  
 or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 =



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(Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =  
5 (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at  
10 res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at  
15 res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56  
20 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg  
25 or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr  
30 or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 =  
35 (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His);

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Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or Arg);

### Generic Sequence 4

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Tyr	Val	Xaa	Phe
10	1					5				10
	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
					15					
	Xaa	Ala	Pro	Xaa	Gly	Xaa	Xaa	Ala		
	20					25				
15	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
			30					35		
	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa			
					40					
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa		
20			45					50		
	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa		
					55					
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys		
	60						65			
25	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
				70						
	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa			
	75					80				
	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa		
30				85						
	Xaa	Xaa	Xaa	Xaa	Met	Xaa	Val	Xaa		
	90					95				
	Xaa	Cys	Gly	Cys	Xaa					
			100							

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wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln; Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp);

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Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

15

Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein family members identified in Table II. Specifically, Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14), human BMP3 (Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq. ID Nos. 24-25). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the variable positions within the sequence. As for Generic

35

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Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 5

```

10      Leu Xaa Xaa Xaa Phe
          1           5
      Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                10
15      Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
          15           20
      Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                25           30
      Xaa Pro Xaa Xaa Xaa Xaa Xaa
20      35
      Xaa Xaa Xaa Asn His Ala Xaa Xaa
                40           45
      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                50
25      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
          55           60
      Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                65

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Xaa Xaa Xaa Leu Xaa Xaa Xaa

70

75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

5 Xaa Xaa Xaa Xaa Met Xaa Val Xaa

85

90

Xaa Cys Xaa Cys Xaa

95

wherein each Xaa is independently selected from a group  
 10 of one or more specified amino acids defined as  
 follows: "Res." means "residue" and Xaa at res.2 =  
 (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4  
 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys  
 or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8  
 15 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp,  
 His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or  
 Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 =  
 (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18  
 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 =  
 20 (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at  
 res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly);  
 Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 =  
 (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu,  
 Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro,  
 25 Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at  
 res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp,  
 Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu,  
 Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or  
 Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at  
 30 res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser,  
 Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at  
 res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu

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or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val);

5 Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 =

10 (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68

15 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or

20 Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln,

25 His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly

30 or Ala) and Xaa at res.97 = (His or Arg).

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Generic Sequence 6

```

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1              5              10
5  Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
    15
    Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
    20              25
10  Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
    30              35
    Xaa Pro Xaa Xaa Xaa Xaa Xaa
    40
    Xaa Xaa Xaa Asn His Ala Xaa Xaa
    45              50
15  Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    55
    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
    60              65
20  Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
    70
    Xaa Xaa Xaa Leu Xaa Xaa Xaa
    75              80
    Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
    85
25  Xaa Xaa Xaa Xaa Met Xaa Val Xaa
    90              95
    Xaa Cys Xaa Cys Xaa
    100

```

30 wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 =

35 (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at



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res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa  
at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg,  
Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or  
Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16  
5 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 =  
(Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val);  
Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or  
Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg);  
Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or  
10 Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln,  
Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at  
res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at  
res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 =  
(Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu  
15 or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at  
res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 =  
(Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,  
Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly  
or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at  
20 res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,  
Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at  
res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or  
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53  
= (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at  
25 res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,  
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,  
Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,  
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at  
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =  
30 (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);  
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro  
or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at  
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);  
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =  
35 (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or

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60A	Ser	...	...	...	...
BMP5	Ser	...	...	...	...
BMP6	...	...	...	...	...
			100		

5

\*\*Between residues 56 and 57 of BMP3 is a Val residue;  
 between residues 43 and 44 of GDF-1 lies  
 the amino acid sequence Gly-Gly-Pro-Pro.

10

As is apparent from the foregoing amino acid  
 sequence comparisons, significant amino acid changes  
 can be made within the generic sequences while  
 retaining the morphogenic activity. For example, while  
 15 the GDF-1 protein sequence depicted in Table II shares  
 only about 50% amino acid identity with the hOP1  
 sequence described therein, the GDF-1 sequence shares  
 greater than 70% amino acid sequence homology (or  
 "similarity") with the hOP1 sequence, where "homology"  
 20 or "similarity" includes allowed conservative amino  
 acid changes within the sequence as defined by Dayoff,  
 et al., Atlas of Protein Sequence and Structure vol.5,  
 supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed.  
 Res. Fd'n, Washington D.C. 1979.)

25

The currently most preferred protein sequences  
 useful as morphogens in this invention include those  
 having greater than 60% identity, preferably greater  
 than 65% identity, with the amino acid sequence  
 30 defining the conserved six cysteine skeleton of hOP1  
 (e.g., residues 43-139 of Seq. ID No. 5). These most  
 preferred sequences include both allelic and species  
 variants of the OP-1 and OP-2 proteins, including the  
 Drosophila 60A protein. Accordingly, in still another  
 35 preferred aspect, the invention includes morphogens

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comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

## II. Formulations and Methods for Administering Therapeutic Agents

15

The morphogens may be provided to an individual by any suitable means, preferably directly (e.g., locally, as by injection to a nerve tissue locus) or systemically (e.g., parenterally or orally). Where the morphogen is to be provided parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (9.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid

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(TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, association of the mature dimer with the pro domain of the morphogen increases solubility of the protein significantly (see Section II.1, below). In fact, the endogenous protein is thought to be transported in this form. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, polybutyrate, tricalcium phosphate, lactide and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen in vivo. Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl acetate copolymer particles,

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osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example,  
5 polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral  
administration may also include glycocholate for buccal  
10 administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration.

Alternatively, the morphogens described herein may  
15 be administered orally. Oral administration of proteins as therapeutics generally is not practiced as most proteins are readily degraded by digestive enzymes and acids in the mammalian digestive system before they can be absorbed into the bloodstream. However, the  
20 morphogens described herein typically are acid stable and protease-resistant (see, for example, U.S. Pat.No. 4,968,590.) In addition, at least one morphogen, OP-1, has been identified in mammary gland extract, colostrum and 57-day milk. Moreover, the OP-1 purified from  
25 mammary gland extract is morphogenically active. Specifically, this protein induces endochondral bone formation in mammals when implanted subcutaneously in association with a suitable matrix material, using a standard in vivo bone assay, such as is disclosed in  
30 U.S. Pat.No. 4,968,590. Moreover, the morphogen also is detected in the bloodstream (see Example 9, below). Finally, soluble form morphogen, e.g., mature morphogen associated with the pro domain, is capable of maintaining neural pathways in a mammal (See Examples 4  
35 and 6 below). These findings indicate that oral and

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parenteral administration are viable means for administering morphogens to an individual. In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

The compounds provided herein also may be associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to nerve tissue. For example, an antibody, antibody fragment, or other binding protein that interacts specifically with a surface molecule on nerve tissue cells, including neuronal or glial cells, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As described above, the morphogens provided herein share significant sequence homology in the C-terminal active domains. By contrast, the sequences typically diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain is thought to be morphogen-specific. As described above, it is also known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the body, selected morphogens typically act on a given

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tissue. Accordingly, part or all of the pro domains which have been identified associated with the active form of the morphogen in solution, may serve as targeting molecules for the morphogens described  
5 herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful targeting molecule for targeting morphogen to nerve  
10 tissue is part or all of a morphogen pro domain, particularly part or all of the pro domains of OP-1 or GDF-1, both of which proteins are found naturally associated with nerve tissue.

15 Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in combination with other molecules known to be beneficial in maintaining neural pathways, including nerve growth factors and anti-inflammatory agents.

20 The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions may be  
25 prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols.

30 The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations for a time  
35 sufficient to eliminate or reduce the patient's

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pathological condition, to provide therapy for the neurological diseases and disorders described above, and amounts effective to enhance neural cell survival an/or to protect neurons and neural pathways in  
5 anticipation of injury to nerve tissue.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a  
10 number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such  
15 variables as the type and extent of progression of the neurological disease, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound excipients, and its route of administration. In general  
20 terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a  
25 preferred dose range is from about 0.1  $\mu$ g/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given in all cases is between 2-20  $\mu$ g of protein per kilogram weight of the patient per day. No obvious OP-1 induced pathological lesions are  
30 induced when mature morphogen (e.g., OP-1, 20  $\mu$ g) is administered daily to normal growing rats for



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21 consecutive days. Moreover, 10 µg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

5

Since the ability of proteins and protein fragments to penetrate the blood-brain barrier may be related to their size, lipophilicity or their net ionic charge, suitable modifications of the morphogens may be formulated (e.g., by substituting pentafluorophenylalanine for phenylalanine, or by conjugation to a cationized protein such as albumin) to increase their transportability across the barrier, using standard methodologies known in the art. See, for example, Kastin et al., Pharmac. Biochem. Behav. (1979) 11:713-716; Rapoport et al., Science (1980) 207:84-86; Pardridge et al., (1987) Biochem. Biophys. Res. Commun. 146:307-313; Riekkinen et al., (1987) Peptides 8:261-265. The efficacy of these functional analogs may be assessed for example, by evaluating the ability of these compounds to induce redifferentiation of transformed cells, or enhance survival of neurons at risk of dying, as described in the Examples provided herein.

25

In administering morphogens systemically in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

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Where injury to neurons of a neural pathway is induced deliberately as part of, for example, a surgical procedure, the morphogen preferably is provided just prior to, or concomitant with induction of the trauma. Preferably, the morphogen is administered prophylactically in a surgical setting. Optimally, the morphogen dosage given in all cases is between 2-20  $\mu$ g of protein per kilogram weight of the patient.

10

Alternatively, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described above. For example, an agent capable of stimulating morphogen production and/or secretion from nerve tissue cells may be provided to a mammal, e.g., by direct administration of the morphogen to glial cells associated with the nerve tissue to be treated. A method for identifying and testing agents capable of modulating the levels of endogenous morphogens in a given tissue is described generally herein in Example 13, and in detail in international application US92/07359 (WO93/015172), the disclosure of which is incorporated herein by reference. Briefly, candidate compounds can be identified and tested by incubating the compound in vitro with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Here, suitable tissue or cultured cells of a tissue preferably would comprise neurons and/or glial cells. For example, suitable tissue for testing may include cultured cells isolated from the substantia nigra, adenoma glial cells, and the like.

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A currently preferred detection means for evaluating the level of the morphogen in culture upon exposure to the candidate compound comprises an immunoassay utilizing an antibody or other suitable binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific for that morphogen. As described herein, morphogens may be isolated from natural-sourced material or they may be recombinantly produced. Agents capable of stimulating endogenous morphogens then may be formulated into pharmaceutical preparations and administered as described herein.

Where the morphogen is to be provided to a site to stimulate axon regeneration, the morphogen preferably is provided to the site in association with a biocompatible, preferably bioresorbable carrier suitable for maintaining a protein at a site in vivo, and through which a neural process may regenerate. A currently preferred carrier also comprises sufficient structure to assist direction of axonal growth. Currently preferred carriers include structural molecules such as collagen, hyaluronic acid or laminin, and/or synthetic polymers or copolymers of, for example, polylactic acid, polyglycolic acid or polybutyric acid. Currently most preferred are carriers comprising tissue extracellular matrix. These may be obtained commercially. In addition, a brain

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tissue-derived extracellular matrix may be prepared as described in international application US92/01968 (W092/15323), incorporated hereinabove by reference, and/or by other means known in the art.

5

The currently preferred means for repairing breaks in neural pathways, particularly pathways of the peripheral nervous system, include providing the morphogen to the site as part of a device that includes a biocompatible membrane or casing of a dimension sufficient to span the break and having openings adapted to receive severed nerve ends. The morphogen is disposed within the casing, preferably dispersed throughout a suitable carrier, and is accessible to the severed nerve ends. Alternatively, the morphogen may be adsorbed onto the interior surface of the casing, or otherwise associated therewith. In addition, currently preferred casings have an impermeable exterior surface. The casing acts as a nerve guidance channel, aiding in directing axonal growth. In addition, the casing also protects the damaged nerve from immunologically-related agents which may assist in scar tissue formation. Suitable channel or casing materials include silicone or bioresorbable materials such as collagen, hyaluronic acid, laminin, polylactic acid, polyglycolic acid, polybutyric acid and the like. Additionally, although the nerve guidance channels described herein generally are tubular in shape, it should be evident to those skilled in the art that various alternative shapes may be employed. The lumen of the guidance channels may, for example, be oval or even square in cross section.

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Moreover the guidance channels may be constructed of two or more parts which may be clamped together to secure the nerve stumps. Nerve endings may be secured to the nerve guidance channels by means of sutures, 5 biocompatible adhesives such as fibrin glue, or other means known in the medical art.

#### II.1 Soluble Morphogen Complexes

10 A currently preferred form of the morphogen useful in therapeutic formulations, having improved solubility in aqueous solutions and consisting essentially of amino acids, is a dimeric morphogenic protein comprising at least the 100 amino acid peptide sequence 15 having the pattern of seven or more cysteine residues characteristic of the morphogen family complexed with a peptide comprising part or all of a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof. Preferably, the 20 dimeric morphogenic protein is complexed with two peptides. Also, the dimeric morphogenic protein preferably is noncovalently complexed with the pro region peptide or peptides. The pro region peptides also preferably comprise at least the N-terminal 25 eighteen amino acids that define a given morphogen pro region. In a most preferred embodiment, peptides defining substantially the full length pro region are used.

30 Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins, as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other

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subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain peptide.

5

As described above, useful pro domains include the full length pro regions, as well as various truncated forms hereof, particularly truncated forms cleaved at proteolytic Arg-Xaa-Xaa-Arg cleavage sites. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is enhanced when the pro region comprises the full length form rather than a truncated form, such as the 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro sequences are those encoding the full length form of the pro region for a given morphogen. Other pro sequences contemplated to have utility include biosynthetic pro sequences, particularly those that incorporate a sequence derived from the N-terminal portion of one or more morphogen pro sequences.

25

As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region may be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

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In another preferred aspect, useful pro region peptides include polypeptide chains comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA or RNA sequence encoding at least the N-terminal eighteen amino acids of the pro region sequence for OP1 or OP2, e.g., nucleotides 136-192 and 152-211 of Seq. ID No. 16' and 20, respectively.

10        A.    Isolation of Soluble morphogen complex from conditioned media or body fluid

Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebro-spinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which

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are anticipated to be isolatable using only minor modifications of the protocol described below. An alternative protocol also envisioned to have utility an immunoaffinity column, created using standard  
5 procedures and, for example, using antibody specific for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column.) Protocols for developing immunoaffinity columns are well described in the art, (see, for example, Guide to  
10 Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly sections VII and XI.)

In this experiment OP-1 was expressed in mammalian  
15 CHO (chinese hamster ovary) cells as described in the art (see, for example, international application US90/05903 (WO91/05802).) The CHO cell conditioned media containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC).  
20 The soluble OP-1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1  
25 from the bulk of the contaminating serum proteins that elute in the flow through and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next applied to an S-Sepharose cation-exchange column equilibrated in 20 mM  $\text{NaPO}_4$  (pH 7.0) with 50 mM NaCl.  
30 This S-Sepharose step serves to further purify and concentrate the soluble OP-1 complex in preparation for the following gel filtration step. The protein was



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applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also may be isolated from one or more body fluids, including serum, cerebro-spinal fluid or  
5 peritoneal fluid.

IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M  $\text{ZnSO}_4$ . The conditioned media was  
10 titrated to pH 7.0 and applied directly to the ZN-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After  
15 loading, the column was washed with equilibration buffer and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex then is eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

20

The 50 mM imidazole eluate containing the soluble OP-1 complex was diluted with nine volumes of 20 mM  $\text{NaPO}_4$  (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM  $\text{NaPO}_4$  (pH 7.0)  
25 with 50 mM NaCl. The S-Sepharose resin was loaded with an equivalent of 800 mL of starting conditioned media per mL of resin. After loading the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM  
30  $\text{NaPO}_4$  (pH 7.0). The 300 mM NaCl pool was further purified using gel filtration chromatography. Fifty mLs of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl.  
35 (pH 7.4). The column was eluted at a flow rate of 5

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mL/minute collecting 10 mL fractions. The apparent molecular of the soluble OP-1 was determined by comparison to protein molecular weight standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with coomassie blue. The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.

The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running the complex-containing fraction from the S-200 or S-200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the

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isolated pro domain from mammalian cell produced OP-1 revealed 2 forms of the pro region, the intact form (beginning at residue 30 of Seq. ID No. 16) and a truncated form, (beginning at residue 48 of Seq. ID No. 16.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 16, all of which are active as demonstrated by the standard bone induction assay.

#### B. In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes may be formulated from purified pro domains and mature dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is decreased in a controlled, preferably step-wise manner, so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl,

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preferably 1-2 M urea of GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations are well described in the art, and details for  
5 developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly section V. Complex formation  
10 also may be aided by addition of one or more chaperone proteins.

#### C. Stability of Soluble Morphogen Complexes

15 The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of means. Currently preferred is by means of a pro region  
20 that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 16 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal portion of other morphogens and are believed  
25 to have particular utility in enhancing complex stability for all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and  
30 betaine); nonionic detergents (e.g., Tween 80 or Nonidet P-120); and carrier proteins (e.g., serum

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albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid;; 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (v/v) nonionic detergent;; and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

### III. Examples

#### 10 Example 1. Identification of Morphogen-Expressing Tissue

Determining the tissue distribution of morphogens may be used to identify different morphogens expressed in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA transcripts) readily are identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

30

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high sequence homology in their active, C-terminal domains,

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- the tissue distribution of a specific morphogen transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein.
- 5 Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon. These portions of the sequence vary substantially among the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly
- 10 useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence).
- 15 Similarly, particularly useful mOP-1-specific probe sequences are the BstXI-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain;
- 20 and the Earl-PstI fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16)
- 25 or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in

30 mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology

35 such as by the method of Chomcyszski et al. ((1987)

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Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15  $\mu$ g) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm<sup>2</sup>). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult tissue are disclosed in international application US92/01968 (WO92/15323), and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commun. 179:116-123, and Ozkaynak, et al. (1992) J. Biol.Chem. 267: 25220-25227. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related tissue appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. Lung tissue appears to be the primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney and heart tissue, while the liver appears to be a

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seen in kidney and heart tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed  
5 primarily in brain tissue. To date, OP-2 appears to be expressed primarily in early embryonic tissue. Specifically, northern blots of murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in  
10 17-day embryos and is not detected in post-natal animals.

Example 2. Morphogen Localization in the Nervous System

15

Morphogens have been identified in developing and adult rat brain and spinal cord tissue, as determined both by northern blot hybridization of morphogen-specific probes to mRNA extracts from developing and  
20 adult nerve tissue (see Example 1, above) and by immunolocalization studies. For example, northern blot analysis of developing rat tissue has identified significant OP-1 mRNA transcript expression in the CNS international application US92/01968 (WO92/15323), and  
25 Ozkaynak et al. (1991) Biochem. Biophys. Res. Comm., 179:11623 and Ozkaynak, et al. (1992) J. Biol. Chem. 267:25220-25227. GDF-1 mRNA appears to be expressed primarily in developing and adult nerve tissue, specifically in the brain, including the cerebellum and  
30 brain stem, spinal cord and peripheral nerves (Lee, S. (1991) PNAS 88: 4250-4254). BMP2B (also referred in the art as BMP4) and Vgr-1 transcripts also have been reported to be expressed in nerve tissue (Jones et al. (1991) Development 111:531-542), although the nerve  
35 tissue does not appear to be the primary expression



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tissue for these genes (Ozkaynak, et al., (1992) J. Biol. Chem. 267:25220-25227. Specifically, CBMP2 transcripts are reported in the region of the diencephalon associated with pituitary development, and  
5 Vgr-1 transcripts are reported in the anteroposterior axis of the CNS, including in the roof plate of the developing neural tube, as well as in the cells immediately adjacent the floor plate of the developing neural tube. In older rats, Vgr-1 transcripts are  
10 reported in developing hippocampus tissue. In addition, the genes encoding OP-1 and BMP2 originally were identified by probing human hippocampus cDNA libraries.

15 Immunolocalization studies, performed using standard methodologies known in the art and disclosed in international application US92/01968 (WO92/15323), the disclosure of which is incorporated herein, localized OP-1 expression to particular areas of  
20 developing and adult rat brain and spinal cord tissue. Specifically, OP-1 protein expression was assessed in adult (2-3 months old) and five or six-day old mouse embryonic nerve tissue, using standard morphogen-specific antisera, specifically, rabbit anti-OP1  
25 antisera, made using standard antibody protocols known in the art and preferably purified on an OP-1 affinity column. The antibody itself was labelled using standard fluorescent labelling techniques, or a labelled anti-rabbit IgG molecule was used to visualize  
30 bound OP-1 antibody.

As can be seen in FIG 1A and 1B, immunofluorescence staining demonstrates the presence of OP-1 in adult rat central nervous system (CNS.) Similar and extensive  
35 staining is seen in both the brain (1A) and spinal cord

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(1B). OP-1 appears to be localized predominantly to the extracellular matrix of the grey matter (neuronal cell bodies), distinctly present in all areas except the cell bodies themselves. In white matter, formed  
5 mainly of myelinated nerve fibers, staining appears to be confined to astrocytes (glial cells). A similar staining pattern also was seen in newborn rat (10 day old) brain sections.

10 In addition, OP-1 has been specifically localized in the substantia nigra, which is composed primarily of striatal basal ganglia, a system of accessory motor neurons that function is association with the cerebral cortex and corticospinal system. Dysfunctions in this  
15 subpopulation or system of neurons are associated with a number of neuropathies, including Huntington's chorea and Parkinson's disease.

OP1 also has been localized at adendema glial  
20 cells, known to secrete factors into the cerebrospinal fluid, and which occur around the intraventricular valve, coroid fissure, and central canal of the brain in both developing and adult rat.

25 Finally, morphogen inhibition in developing embryos inhibits nerve tissue development. Specifically, 9-day mouse embryo cells, cultured in vitro under standard culturing conditions, were incubated in the presence and absence of an OP-1-specific monoclonal antibody  
30 prepared using recombinantly produced, purified mature OP-1 and the immunogen. The antibody was prepared using standard antibody production means well known in the art and as described generally in Example 13. After two days, the effect of the antibody on the  
35 developing embryo was evaluated by histology. As

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determined by histological examination, the OP-1-specific antibody specifically inhibits eye lobe formation in the developing embryo. In particular, the diencephalon outgrowth does not develop. In addition, the heart is malformed and enlarged. Moreover, in separate immunolocalization studies on embryo sections with labelled OP-1 specific antibody, the OP-1-specific antibody localizes to neural epithelia.

The endogenous morphogens which act on neuronal cells may be expressed and secreted by nerve tissue cells, e.g., by neurons and/or glial cells associated with the neurons, and/or they may be transported to the neurons by the cerebrospinal fluid and/or bloodstream. Recently, OP-1 has been identified in the human blood (See Example 9, below). In addition, transplanted Schwann cells recently have been shown to stimulate nerve fiber formation in rat spinal cord, including inducing vascularization and myelin sheath formation around at least some of the new neuronal processes (Bunge (1991) Exp. Neurology 114:254-257.) The regenerative property of these cells may be mediated by the secretion of a morphogen by the Schwann cells.

Example 3. Morphogen Enhancement of Neuronal Cell Survival

The morphogens described herein enhance cell survival, particularly of neuronal cells at risk of dying. For example, fully differentiated neurons are non-mitotic and die in vitro when cultured under standard mammalian cell culture conditions, using a chemically defined or low serum medium known in the art, (see, for example, Charness (1986) J. Biol. Chem. 26:3164-3169 and Freese et al. (1990) Brain Res.

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521:254-264.) However, if a primary culture of non-mitotic neuronal cells is treated with a morphogen, the survival of these cells is enhanced significantly. For example, a primary culture of striatal basal ganglia isolated from the substantia nigra of adult rat brain was prepared using standard procedures, e.g., by dissociation by trituration with pasteur pipette of substantia nigra tissue, using standard tissue culturing protocols, and grown in a low serum medium, e.g., containing 50% DMEM (Dulbecco's modified Eagle's medium), 50% F-12 medium, heat inactivated horse serum supplemented with penicillin/streptomycin and 4 g/l glucose. Under standard culture conditions, these cells are undergoing significant cell death by three weeks when cultured in a serum-free medium. Cell death is evidenced morphologically by the inability of cells to remain adherent and by changes in their ultrastructural characteristics, e.g., by chromatin clumping and organelle disintegration.

20

In this example, the cultured basal ganglia were treated with chemically defined medium conditioned with 0.1-100 ng/ml OP-1. Fresh, morphogen-conditioned medium was provided to the cells every 3-4 days. Cell survival was enhanced significantly and was dose dependent upon the level of OP-1 added: cell death decreased significantly as the concentration of OP-1 was increased in cell cultures. Specifically, cells remained adherent and continued to maintain the morphology of viable differentiated neurons. In the absence of morphogen treatment, the majority of the cultured cells dissociated and underwent cell necrosis.

30

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Dysfunctions in the basal ganglia of the substantia nigra are associated with Huntington's chorea and parkinsonism in vivo. The ability of the morphogens defined herein to enhance neuron survival indicates  
5 that these morphogens will be useful as part of a therapy to enhance survival of neuronal cells at risk of dying in vivo due, for example, to a neuropathy or chemical or mechanical trauma. It is particularly anticipated that these morphogens will provide a useful  
10 therapeutic agent to treat neuropathies which affect the striatal basal ganglia, including Huntington's chorea and Parkinson's disease. For clinical applications, the morphogen may be administered or, alternatively, a morphogen-stimulating agent may be  
15 administered.

Example 4. Morphogen-Induced Redifferentiation of Transformed Cells

20

The morphogens described herein also induce redifferentiation of transformed cells to a morphology characteristic of untransformed cells. In particular, the morphogens are capable of inducing  
25 redifferentiation of transformed cells of neuronal origin to a morphology characteristic of untransformed neurons. The example provided below details morphogen induced redifferentiation of a transformed human cell line of neuronal origin, NG105-115. Morphogen-induced  
30 redifferentiation of transformed cells also has been shown in mouse neuroblastoma cells (N1E-115) and in human embryo carcinoma cells (see international application US92/01968 (WO92/15323)).

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NG108-15 is a transformed hybrid cell line produced by fusing neuroblastoma x glioma cells (obtained from America Type Tissue Culture, Rockville, MD), and exhibiting a morphology characteristic of transformed embryonic neurons, e.g., having a fibroblastic morphology. Specifically, the cells have polygonal cell bodies, short, spike-like processes and make few contacts with neighboring cells (see FIG. 1A). Incubation of NG108-15 cells, cultured in a chemically defined, serum-free medium, with 0.1 to 300 ng/ml of OP-1 for four hours induces an orderly, dose-dependent change in cell morphology.

In the experiment NG108-15 cells were subcultured on poly-L-lysine coated 6-well plates. Each well contained 40-50,000 cells in 2.5 ml of chemically defined medium. On the third day 2.5  $\mu$ l of OP-1 in 60% ethanol containing 0.025% trifluoroacetic was added to each well. OP-1 concentrations of 0-300 ng/ml were tested. Typically, the media was changed daily with new aliquots of OP-1, although morphogenesis can be induced by a single four hour incubation with OP-1. In addition, OP-1 concentrations of 10 ng/ml were sufficient to induce redifferentiation. After one day, hOP-1-treated cells undergo a significant change in their cellular ultrastructure, including rounding of the soma, increase in phase brightness and extension of the short neurite processes. After two days, cells treated with OP-1 begin to form epithelioid sheets, which provide the basis for the growth of multilayered aggregates at three day's post-treatment. By four days, the great majority of OP-1-treated cells are associated in tightly-packed, multilayered aggregates

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(Fig. 1B). Fig. 2 plots the mean number of multi-layered aggregates or cell clumps identified in twenty randomly selected fields from six independent experiments, as a function of morphogen concentration.

- 5 Forty ng/ml of OP-1 is sufficient for half maximum induction of cell aggregation.

The morphogen-induced redifferentiation occurred without any associated changes in DNA synthesis, cell  
10 division, or cell viability, making it unlikely that the morphologic changes were secondary to cell differentiation or a toxic effect of hOP-1. Moreover, the OP-1-induced morphogenesis does not inhibit cell division, as determined by <sup>3</sup>H-thymidine uptake, unlike  
15 other molecules which have been shown to stimulate differentiation of transformed cells, such as butyrate, DMSO, retanoic acid or Forskolin. The data indicate that OP-1 can maintain cell stability and viability after inducing redifferentiation. In addition, the  
20 effects are morphogen specific, and redifferentiation is not induced when NG108-15 cells are incubated with 0.1-40 ng/ml TGF- $\beta$ .

The experiments also have been performed with  
25 highly purified soluble morphogen (e.g., mature OP1 associated with its pro domain) which also specifically induced redifferentiation of NG108-15 cells.

The morphogens described herein accordingly provide  
30 useful therapeutic agents for the treatment of neoplasias and neoplastic lesions of the nervous system, particularly in the treatment of

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neuroblastomas, including retinoblastomas, and gliomas. The morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

5

Example 5. Nerve Tissue Protection from Chemical Trauma

10 The ability of the morphogens described herein to enhance survival of neuronal cells and to induce cell aggregation and cell-cell adhesion in redifferentiated cells, indicates that the morphogens will be useful as  
15 therapeutic agents to maintain neural pathways by protecting the cells defining the pathway from the damage caused by chemical trauma. In particular, the morphogens can protect neurons, including developing neurons, from the effects of toxins known to inhibit the proliferation and migration of neurons and to  
20 interfere with cell-cell adhesion. Examples of such toxins include ethanol, one or more of the toxins present in cigarette smoke, and a variety of opiates. The toxic effects of ethanol on developing neurons induces the neurological damage manifested in fetal  
25 alcohol syndrome. The morphogens also may protect neurons from the cytotoxic effects associated with excitatory amino acids such as glutamate.

For example, ethanol inhibits the cell-cell  
30 adhesion effects induced in morphogen-treated NG108-15 cells when provided to these cells at a concentration of 25-50 mM. Half maximal inhibition can be achieved with 5-10 mM ethanol, the concentration of blood alcohol in an adult following ingestion of a single  
35 alcoholic beverage. Ethanol likely interferes with the



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homophilic binding of CAMs between cells, rather than their induction, as morphogen-induced N-CAM levels are unaffected by ethanol. Moreover, the inhibitory effect is inversely proportional to morphogen concentration.

5 Accordingly, it is envisioned that administration of a morphogen or morphogen-stimulating agent to neurons, particularly developing neurons, at risk of damage from exposure to toxins such as ethanol, may protect these cells from nerve tissue damage by overcoming the  
10 toxin's inhibitory effects. The morphogens described herein also are useful in therapies to treat damaged neural pathways resulting from a neuropathy induced by exposure to these toxins.

15

Example 6. Morphogen-Induced CAM Expression

The morphogens described herein induce CAM expression, particularly N-CAM expression, as part of  
20 their induction of morphogenesis. CAMs are morphoregulatory molecules identified in all tissues as an essential step in tissue development. N-CAMs, which comprise at least 3 isoforms (N-CAM-180, N-CAM-140 and N-CAM-120, where "180", "140" and "120" indicate the  
25 apparent molecular weights of the isoforms as measured by polyacrylamide gel electrophoresis) are expressed at least transiently in developing tissues, and permanently in nerve tissue. Both the N-CAM-180 and N-CAM-140 isoforms are expressed in both developing and  
30 adult tissue. The N-CAM-120 isoform is found only in adult tissue. Another neural CAM is L1.

N-CAMs are implicated in appropriate neural development, including appropriate nuerulation,  
35 neuronal migration, fasciculation, and synaptogenesis.

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Inhibition of N-CAM production, as by complexing the molecule with an N-CAM-specific antibody, inhibits retina organization, including retinal axon migration, and axon regeneration in the peripheral nervous system, as well as axon synapsis with target muscle cells. In addition, significant evidence indicates that physical or chemical trauma to neurons, oncogenic transformation and some genetic neurological disorders are accompanied by changes in CAM expression, which alter the adhesive or migratory behavior of these cells. Specifically, increased N-CAM levels are reported in Huntington's disease striatum (e.g., striatal basal ganglia), and decreased adhesion is noted in Alzheimer's disease.

15       The morphogens described herein can stimulate CAM production, particularly L1 and N-CAM production, including all three isoforms of the N-CAM molecule. For example, N-CAM expression is stimulated significantly in morphogen-treated NG108-15 cells.

20       Untreated NG108-15 cells exhibit a fibroblastic, or minimally differentiated morphology and express only the 180 and 140 isoforms of N-CAM normally associated with a developing cell. Following morphogen treatment these cells exhibit a morphology characteristic of

25       adult neurons and express enhanced levels of all three N-CAM isoforms. Using a similar protocol as described in the example below, morphogen treatment of NG108-15 cells also induced L1 expression.

30       In this example NG108-15 cells were cultured for 4 days in the presence of increasing concentrations of OP-1 and standard Western blots performed on whole cells extracts. N-CAM isoforms were detected with an antibody which crossreacts with all three isoforms,

35       mAb H28.123, obtained from Sigma Chemical Co.,

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St. Louis, the different isoforms being distinguishable by their different mobilities on an electrophoresis gel. Control NG108-15 cells (untreated) express both the 140 kDa and the 180 kDa isoforms, but not the 120 kDa, as determined by western blot analyses using up to 100  $\mu$ g of protein. Treatment of NG108-15 cells with OP-1 resulted in a dose-dependent increase in the expression of the 180 kDa and 140 kDa isoforms, as well as the induction of the 120 kDa isoform. See Fig. 2A and 2B. Fig. 2B is a Western blot of OP1-treated NG108-15 cell extracts, probed with mAb H28.123, showing the induction of all three isoforms. Fig. 2A is a dose response curve of N-CAM-180 and N-CAM-140 induction as a function of morphogen concentration. N-CAM-120 is not shown in the graph as it could not be quantitated in control cells. However, as is clearly evident from the Western blot in Fig. 2A, N-CAM-120 is induced in response to morphogen treatment. The differential induction of N-CAM 180 and 140 isoforms seen may be because constitutive expression of the 140 isoform is close to maximum.

The increase in N-CAM expression corresponded in a dose-dependent manner with the morphogen induction of multicellular aggregates. Compare Fig. 2A and Fig 3. Fig. 3 graphs the mean number of multilayered aggregates (clumps) counted per 20 randomly selected fields in 6 independent experiments, versus the concentration of morphogen. The induction of the 120 isoform also indicates that morphogen-induced redifferentiation of transformed cells stimulates not only redifferentiation of these cells from a transformed phenotype, but also differentiation to a phenotype corresponding to a developed cell. Standard immunolocalization studies performed with the mAb

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H28.123 on morphogen-treated cells show N-CAM cluster formation associated with the periphery and processes of treated cells and no reactivity with untreated cells. Moreover, morphogen treatment does not appear to inhibit cell division as determined by cell counting or <sup>3</sup>H-thymidine uptake. Finally, known chemical differentiating agents, such as Forskolin and dimethylsulfoxide do not induce N-CAM production.

10 In addition, the cell aggregation effects of OP-1 on NG108-15 cells can be inhibited with anti-N-CAM antibodies or antisense N-CAM oligonucleotides. Antisense oligonucleotides can be made synthetically on a nucleotide synthesizer, using standard means known in the art. Preferably, phosphorothioate oligonucleotides ("S-oligos") are prepared, to enhance transport of the nucleotides across cell membranes. Concentrations of both N-CAM antibodies and N-CAM antisense oligonucleotides sufficient to inhibit N-CAM induction also inhibited formation of multilayered cell aggregates. Specifically, incubation of morphogen-treated NG108-115 cells with 0.3-3  $\mu$ M N-CAM antisense S-oligos, 5-500  $\mu$ M unmodified N-CAM antisense oligos, or 10  $\mu$ g/ml mAb H28.123 significantly inhibits cell aggregation. It is likely that morphogen treatment also stimulates other CAMs, as inhibition is not complete.

30 The experiments also have been performed with soluble morphogen (e.g., mature OP-1 associated with its pro domain) which also specifically induced CAM expression.

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The morphogens described herein are useful as therapeutic agents to treat neurological disorders associated with altered CAM levels, particularly N-CAM levels, such as Huntington's chorea and Alzheimers' disease, and the like. In clinical applications, the morphogens themselves may be administered or, alternatively, a morphogen-stimulating agent may be administered.

10 The efficacy of the morphogens described herein to affect N-CAM expression may be assessed in vitro using a suitable cell line and the methods described herein. In addition to a transformed cell line, N-CAM expression can be assayed in a primary cell culture of  
15 neural or glial cells, following the procedures described herein. The efficacy of morphogen treatment on N-CAM expression in vivo may be evaluated by tissue biopsy as described in Example 9, below, and detecting N-CAM molecules with an N-CAM-specific antibody, such  
20 as mAb H28.123, or using the animal model described in Example 11.

Alternatively, the level of N-CAM proteins or protein fragments present in cerebrospinal fluid or  
25 serum also may be detected to evaluate the effect of morphogen treatment. N-CAM molecules are known to slough off cell surfaces and have been detected in both serum and cerebrospinal fluid. In addition, altered levels of the soluble form of N-CAM are associated with  
30 normal pressure hydrocephalus and type II schizophrenia. N-CAM fluid levels may be detected following the procedure described in Example 9 and using an N-CAM specific antibody, such as mAb H28.123.

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**Example 7. Morphogen-Induced Nerve Gap Repair (PNS)**

The morphogens described herein also stimulate peripheral nervous system axonal growth over extended distances allowing repair and regeneration of damaged neural pathways. While neurons of the peripheral nervous system can sprout new processes following injury, without guidance these sproutings typically fail to connect appropriately and die. Where the break is extensive, e.g., greater than 5 or 10 mm, regeneration is poor or nonexistent.

In this example morphogen stimulation of nerve regeneration was assessed using the rat sciatic nerve model. The rat sciatic nerve can regenerate spontaneously across a 5 mm gap, and occasionally across a 10 mm gap, provided that the severed ends are inserted in a saline-filled nerve guidance channel. In this experiment, nerve regeneration across a 12mm gap was tested.

Adult female Sprague-Dawley rats (Charles River Laboratories, Inc.) weighing 230-250 g were anesthetized with intraperitoneal injections of sodium pentobarbital 35 mg/kg body weight). A skin incision was made parallel and just posterior to the femur. The avascular intermuscular plane between vastus lateralis and hamstring muscles were entered and followed to the loose fibroareolar tissue surrounding the sciatic nerve. The loose tissue was divided longitudinally thereby freeing the sciatic nerve over its full extent without devascularizing any portion. Under a surgical

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microscope the sciatic nerves were transected with microscissors at mid-thigh and grafted with an OP-1 gel graft that separated the nerve stumps by 12 mm. The graft region was encased in a silicone tube 20 mm in length with a 1.5 mm inner diameter, the interior of which was filled a morphogen solution. Specifically, The central 12 mm of the tube consisted of an OP-1 gel prepared by mixing 1 to 5  $\mu$ g of substantially pure CHO-produced recombinant OP-1 with approximately 100  $\mu$ l of MATRIGEL<sup>TM</sup> (from Collaborative Research, Inc., Bedford, MA), an extracellular matrix extract derived from mouse sarcoma tissue, and containing solubilized tissue basement membrane, including laminin, type IV collagen, heparin sulfate, proteoglycan and entactin, in phosphate-buffered saline. The OP-1-filled tube was implanted directly into the defect site, allowing 4 mm on each end to insert the nerve stumps. Each stump was abutted against the OP-1 gel and was secured in the silicone tube by three stitches of commercially available surgical 10-0 nylon through the epineurium, the fascicle protective sheath.

In addition to OP-1 gel grafts, empty silicone tubes, silicone tubes filled with gel only and "reverse" autografts, wherein 12 mm transected segments of the animal's sciatic nerve were rotated 180° prior to suturing, were grafted as controls. All experiments were performed in quadruplicate. All wounds were closed by wound clips that were removed after 10 days. All rats were grafted on both legs. At 3 weeks the animals were sacrificed, and the grafted segments removed and frozen on dry ice immediately. Frozen

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sections then were cut throughout the graft site, and examined for axonal regeneration by immunofluorescent staining using anti-neurofilament antibodies labeled with flurocein (obtained from Sigma Chemical Co.,  
5 St. Louis).

Regeneration of the sciatic nerve occurred across the entire 12 mm distance in all graft sites wherein the gap was filled with the OP-1 gel. By contrast,  
10 empty silicone tubes and reverse autografts did not show nerve regeneration, and only one graft site containing the gel alone showed axon regeneration.

15 **Example 8. Morphogen-Induced Nerve Gap Repair (CNS)**

Following axonal damage in vivo the CNS neurons are unable to resprout processes. Accordingly, trauma to CNS nerve tissue, including the spinal cord, optic  
20 nerve and retina, severely damages or destroys the neural pathways defined by these cells. Peripheral nerve grafts have been used in an effort to bypass CNS axonal damage. Successful autologous graft repair to date apparently requires that the graft site occur near  
25 the CNS neuronal cell body, and a primary result of CNS axotomy is neuronal cell death. The efficacy of morphogens described herein on CNS nerve repair, may be evaluated using a rat crushed optic nerve model such as the one described by Bignami et al., (1979) Exp. Eye  
30 Res. 28: 63-69, the disclosure of which is incorporated herein by reference. Briefly, and as described therein, laboratory rats (e.g., from Charles River Laboratories, Wilmington, MA) are anesthetized using standard surgical procedures, and the optic nerve  
35 crushed by pulling the eye gently out of the orbit,



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inserting a watchmaker forceps behind the eyeball and squeezing the optic nerve with the forceps for 15 seconds, followed by a 30 second interval and second 15 second squeeze. Rats are sacrificed at different 5 time intervals, e.g., at 48 hours, and at 3, 4, 11, 15 and 18 days after operation. The effect of morphogen on optic nerve repair can be assessed by performing the experiment in duplicate and providing morphogen or PBS (e.g., 25  $\mu$ l solution, and 25  $\mu$ g morphogen) to the 10 optic nerve, e.g., just prior to the operation, concomitant with the operation, or at specific times after the operation.

In the absence of therapy, the surgery induces 15 glial scarring of the crushed nerve, as determined by immunofluorescence staining for glial fibrillary acidic protein (GFA), a marker protein for glial scarring, and by histology. Indirect immunofluorescence on air-dried cryostat sections as described in Bignami et al. (1974) 20 J. Comp. Neur. 153: 27-38, using commercially available antibodies to GFA (e.g., Sigma Chemical Co., St. Louis). Reduced levels of GFA are anticipated in animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation 25 and to stimulate optic nerve regeneration.

#### Example 9. Nerve Tissue Diagnostics

Morphogen localization in nerve tissue can be used 30 as part of a method for diagnosing a neurological disorder or neuropathy. The method may be particularly advantageous for diagnosing neuropathies of brain tissue. Specifically, a biopsy of brain tissue is performed on a patient at risk, using standard 35 procedures known in the medical art. Morphogen

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expression associated with the biopsied tissue then is assessed using standard methodologies, as by immunolocalization, using standard immunofluorescence techniques in concert with morphogen-specific antisera or monoclonal antibodies. Specifically, the biopsied tissue is thin sectioned using standard methodologies known in the art, and fluorescently labelled (or otherwise detectable) antibodies incubated with the tissue under conditions sufficient to allow specific antigen-antibody complex formation. The presence and quantity of complex formed then is detected and compared with a predetermined standard or reference value. Detection of altered levels of morphogen present in the tissue then may be used as an indicator of tissue dysfunction. Alternatively, fluctuation in morphogen levels may be assessed by monitoring morphogen transcription levels, either by standard northern blot analysis or in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen RNA and standard RNA hybridization protocols well described in the art.

Fluctuations in morphogen levels present in the cerebrospinal fluid or bloodstream also may be used to evaluate nerve tissue viability. For example, morphogens are detected associated with adendema cells which are known to secrete factors into the cerebrospinal fluid, and are localized generally associated with glial cells, and in the extracellular matrix, but not with neuronal cell bodies.

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Accordingly, the cerebrospinal fluid may be a natural means of morphogen transport. Alternatively, morphogens may be released from dying cells into cerebrospinal fluid. In addition, OP-1 recently has  
5 been identified in human blood, which also may be a means of morphogen transport, and/or a repository for the contents of dying cells.

Spinal fluid may be obtained from an individual by  
10 a standard lumbar puncture, using standard methodologies known in the medical art. Similarly, serum samples may be obtained by standard venipuncture and serum prepared by centrifugation at 3,000 RPM for ten minutes. The presence of morphogen in the serum or  
15 cerebral spinal fluid then may be assessed by standard Western blot (immunoblot), ELISA or RIA procedures. Briefly, for example, with the ELISA, samples may be diluted in an appropriate buffer, such as phosphate-buffered saline, and 50  $\mu$ l aliquots allowed to absorb  
20 to flat bottomed wells in microtitre plates pre-coated with morphogen-specific antibody, and allowed to incubate for 18 hours at 4°C. Plates then may be washed with a standard buffer and incubated with 50  $\mu$ l aliquots of a second morphogen-specific antibody  
25 conjugated with a detecting agent, e.g., biotin, in an appropriate buffer, for 90 minutes at room temperature. Morphogen-antibody complexes then may be detected using standard procedures.

30 Alternatively, a morphogen-specific affinity column may be created using, for example, morphogen-specific antibodies adsorbed to a column matrix, and passing the fluid sample through the matrix to selectively extract the morphogen of interest. The morphogen then is  
35 eluted. A suitable elution buffer may be determined

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empirically by determining appropriate binding and elution conditions, first with a control (e.g., purified, recombinantly-produced morphogen.) Fractions then are tested for the presence of the morphogen by  
5 standard immunoblot, and confirmed by N-terminal sequencing. Morphogen concentrations in serum or other fluid samples then may be determined using standard protein quantification techniques, including by spectrophotometric absorbance or by quantitation by  
10 ELISA or RIA antibody assays. Using this procedure, OP-1 has been identified in serum.

OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against  
15 mammalian, recombinantly produced OP-1 using standard immunology techniques well described in the art and described generally in Example 13, was immobilized by passing the antibody over an activated agarose gel (e.g., Affi-Gel<sup>TM</sup>, from Bio-Rad Laboratories, Richmond,  
20 CA, prepared following manufacturer's instructions), and used to purify OP-1 from serum. Human serum then was passed over the column and eluted with 3M K-thiocyanate. K-thiocyanate fractions then were dialyzed in 6M urea, 20mM PO<sub>4</sub>, pH 7.0, applied to a C8  
25 HPLC column, and eluted with a 20 minute, 25-50% acetonitrile/0.1% TFA gradient. Mature, recombinantly produced OP-1 homodimers elute between 20-22 minutes. Fractions then were collected and tested for the presence of OP-1 by standard immunoblot. Fig. 4 is an  
30 immunoblot showing OP-1 in human sera under reducing and oxidized conditions. In the figure, lanes 1 and 4 are OP-1 standards, run under oxidized (lane 1) and

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reduced (lane 4) conditions. Lane 5 shows molecular weight markers at 17, 27 and 39 kDa. Lanes 2 and 3 are human sera OP-1, run under oxidized (lane 2) and reduced (lane 3) conditions.

5

Morphogens may be used in diagnostic applications by comparing the quantity of morphogen present in a body fluid sample with a predetermined reference value, with fluctuations in fluid morphogen levels indicating a change in the status of nerve tissue. Alternatively, fluctuations in the level of endogenous morphogen antibodies may be detected by this method, most likely in serum, using an antibody or other binding protein capable of interacting specifically with the endogenous morphogen antibody. Detected fluctuations in the levels of the endogenous antibody may be used as indicators of a change in tissue status.

20 Example 10. Alleviation of Immune Response-Mediated Nerve Tissue Damage

The morphogens described herein may be used to alleviate immunologically-related damage to nerve tissue. Details of this damage and the use of morphogens to alleviate this injury are disclosed in international application US92/07358 (WO93/04692). A primary source of such damage to nerve tissue follows hypoxia or ischemia-reperfusion of a blood supply to a neural pathway, such as may result from an embolic stroke, or may be induced during a surgical procedure.

30

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As described in international application US92/07358 (WO93/04692), morphogens have been shown to alleviate damage to myocardial tissue following ischemia-reperfusion of the blood supply to the tissue. The effect of morphogens on alleviating immunologically-related damage to nerve tissue may be assessed using methodologies and models known to those skilled in the art and described below.

- 10 For example, the rabbit embolic stroke model provides a useful method for assessing the effect of morphogens on tissue injury following cerebral ischemia-reperfusion. The protocol disclosed below is essentially that of Phillips et al. (1989) Annals of  
15 Neurology 25:281-285, the disclosure of which is herein incorporated by reference. Briefly, white New England rabbits (2-3kg) are anesthetized and placed on a respirator. The intracranial circulation then is selectively catheterized by the Seldinger technique.  
20 Baseline cerebral angiography then is performed, employing a digital substration unit. The distal internal carotid artery or its branches then is selectively embolized with 0.035 ml of 18-hour-aged autologous thrombus. Arterial occlusion is documented  
25 by repeat angiography immediately after embolization. After a time sufficient to induce cerebral infarcts (15 minutes or 90 minutes), reperfusion is induced by administering a bolus of a reperfusion agent such as the TPA analogue FB-FB-CF (e.g., 0.8 mg/kg over 2  
30 minutes).

The effect of morphogen on cerebral infarcts can be assessed by administering varying concentrations of morphogens, e.g., OP-1, at different times following  
35 embolization and/or reperfusion. The rabbits are

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sacrificed 3-14 days post embolization and their brains prepared for neuropathological examination by fixing by immersion in 10% neutral buffered formalin for at least 2 weeks. The brains then are sectioned in a coronal plane at 2-3 mm intervals, numbered and submitted for standard histological processing in paraffin, and the degree of nerve tissue necrosis determined visually. Morphogen-treated animals are anticipated to reduce or significantly inhibit nerve tissue necrosis following cerebral ischemia-reperfusion in the test animals as determined by histology comparison with nontreated animals.

Example 11. Animal Model for Assessing Morphogen Efficacy In Vivo

The in vivo activities of the morphogens described herein also are assessed readily in an animal model as described herein. A suitable animal, preferably exhibiting nerve tissue damage, for example, genetically or environmentally induced, is injected intracerebrally with an effective amount of a morphogen in a suitable therapeutic formulation, such as phosphate-buffered saline, pH 7. The morphogen preferably is injected within the area of the affected neurons. The affected tissue is excised at a subsequent time point and the tissue evaluated morphologically and/or by evaluation of an appropriate biochemical marker (e.g., by morphogen or N-CAM localization; or by measuring the dose-dependent effect on a biochemical marker for CNS neurotrophic activity or for CNS tissue damage, using for example, glial fibrillary acidic protein as the marker. The dosage

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and incubation time will vary with the animal to be tested. Suitable dosage ranges for different species may be determined by comparison with established animal models. Presented below is an exemplary protocol for  
5 a rat brain stab model.

Briefly, male Long Evans rats, obtained from standard commercial sources, are anesthetized and the head area prepared for surgery. The calvariae is  
10 exposed using standard surgical procedures and a hole drilled toward the center of each lobe using a 0.035K wire, just piercing the calvariae. 25 $\mu$ l solutions containing either morphogen (e.g., OP-1, 25 $\mu$ g) or PBS then is provided to each of the holes by Hamilton  
15 syringe. Solutions are delivered to a depth approximately 3 mm below the surface, into the underlying cortex, corpus callosum and hippocampus. The skin then is sutured and the animal allowed to recover.

20

Three days post surgery, rats are sacrificed by decapitation and their brains processed for sectioning. Scar tissue formation is evaluated by immunofluorescence staining for glial fibrillary acidic protein, a marker  
25 protein for glial scarring, to qualitatively determine the degree of scar formation. Glial fibrillary acidic protein antibodies are available commercially, e.g., from Sigma Chemical Co., St. Louis, MO. Sections also are probed with anti-OP-1 antibodies to determine the  
30 presence of OP-1. Reduced levels of glial fibrillary acidic protein are anticipated in the tissue sections of animals treated with the morphogen, evidencing the ability of morphogens to inhibit glial scar formation and stimulate nerve regeneration.

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Example 12. In Vitro Model for Evaluating Morphogen Species Transport Across the Blood-Brain Barrier.

5 Described below is an in vitro method for evaluating the facility with which selected morphogen species likely will pass across the blood-brain barrier. A detailed description of the model and protocol are provided by Audus et al. (1987) Ann. N.Y.  
10 Acad. Sci. 507:9-18, the disclosure of which is incorporated herein by reference.

Briefly, microvessel endothelial cells are isolated from the cerebral gray matter of fresh bovine brains.  
15 Brains are obtained from a local slaughter house and transported to the laboratory in ice cold minimum essential medium (MEM) with antibiotics. Under sterile conditions the large surface blood vessels and meninges are removed using standard dissection procedures. The  
20 cortical gray matter is removed by aspiration, then minced into cubes of about 1mm. The minced gray matter then is incubated with 0.5% dispase (BMB, Indianapolis, IN) for 3 hours at 37° C in a shaking water bath. Following the 3 hour digestion, the mixture is  
25 concentrated by centrifugation (1000 x g for 10 min.), then resuspended in 13% dextran and centrifuged for 10 min. at 5800 x g. Supernatant fat, cell debris and myelin are discarded and the crude microvessel pellet resuspended in 1 mg/ml collagenase/dispase and  
30 incubated in a shaking water bath for 5 hours at 37° C. After the 5-hour digestion, the microvessel suspension is applied to a pre-established 50% Percoll gradient and centrifuged for 10 min at 1000 x g. The band containing purified endothelial cells (second band from  
35 the top of the gradient) is removed and washed two

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times with culture medium (e.g., 50% MEM/50% F-12 nutrient mix). The cells are frozen ( -80° C.) in medium containing 20% DMSO and 10% horse serum for later use.

5

After isolation, approximately  $5 \times 10^5$  cells/cm<sup>2</sup> are plated on culture dishes or 5-12  $\mu$  pore size polycarbonate filters that are coated with rat collagen and fibronectin. 10-12 days after seeding the cells, cell monolayers are inspected for confluency by microscopy.

Characterization of the morphological, histochemical and biochemical properties of these cells has shown that these cells possess many of the salient features of the blood-brain barrier. These features include: tight intercellular junctions, lack of membrane fenestrations, low levels of pinocytotic activity, and the presence of gamma-glutamyl transpeptidase, alkaline phosphatase, and Factor VIII antigen activities.

The cultured cells can be used in a wide variety of experiments where a model for polarized binding or transport is required. By plating the cells in multi-well plates, receptor and non-receptor binding of both large and small molecules can be conducted. In order to conduct transendothelial cell flux measurements, the cells are grown on porous polycarbonate membrane filters (e.g., from Nucleopore, Pleasanton, CA). Large pore size filters (5-12  $\mu$ ) are

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used to avoid the possibility of the filter becoming the rate-limiting barrier to molecular flux. The use of these large-pore filters does not permit cell growth under the filter and allows visual inspection of the cell monolayer.

Once the cells reach confluency, they are placed in a side-by-side diffusion cell apparatus (e.g., from Crown Glass, Sommerville, NJ). For flux measurements, the donor chamber of the diffusion cell is pulsed with a test substance, then at various times following the pulse, an aliquot is removed from the receiver chamber for analysis. Radioactive or fluorescently-labelled substances permit reliable quantitation of molecular flux. Monolayer integrity is simultaneously measured by the addition of a non-transportable test substance such as sucrose or inulin and replicates of at least 4 determinations are measured in order to ensure statistical significance.

Example 13. Screening Assay for Candidate Compounds which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to affect the level of a given morphogen may be found using the following screening assay, in which the level of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating the cell in culture with the compound, in order to assess the effects of the compound on the cell. This can be accomplished by detection of the morphogen either at the protein or RNA level. A more detailed description also may be found in international application US92/07359 (WO92/05172).

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### 13.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described  
5 widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from  
10 kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be  
15 cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or  
20 other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1  
25 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor de  
30 novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an <sup>35</sup>S-methionine/<sup>35</sup>S-cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.

35

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### 13.2 Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

- 10        1  $\mu$ g/100  $\mu$ l of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1%
- 15        Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100  $\mu$ l aliquot of an
- 20        appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100  $\mu$ l biotinylated rabbit anti-OP-1 serum
- 25        (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20.
- 30        100  $\mu$ l streptavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline

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(TBS), pH 7.2. 50 $\mu$ l substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well incubated at room temperature for 15 min. Then, 50  $\mu$ l amplifier (from the same  
5 amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50  $\mu$ l 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1  
10 standard curve is performed in parallel with the test samples.

Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100  
15 ug/500  $\mu$ l E. coli produced OP-1 monomer (amino acids 328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500  $\mu$ l Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in  
20 the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against OP-1 is detected in the serum using an ELISA assay.  
25 Then, the rabbit is boosted monthly with 100  $\mu$ g of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

Monoclonal antibody specific for a given morphogen  
30 may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer. The first injection contains 100 $\mu$ g of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50  $\mu$ g of OP-1 in incomplete adjuvant  
35 and is given intraperitoneally. The mouse then

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receives a total of 230  $\mu$ g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, both mice are boosted

5 intraperitoneally with 100  $\mu$ g of OP-1 (307-431) and 30  $\mu$ g of the N-terminal peptide (Ser<sub>293</sub>-Asn<sub>309</sub>-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days

10 (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boehringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as

15 antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

The invention may be embodied in other specific

20 forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather

25 than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

## (i) APPLICANT:

- (A) NAME: CREATIVE BIOMOLECULES, INC.
- (B) STREET: 35 SOUTH STREET
- (C) CITY: HOPKINTON
- 10 (D) STATE: MASSACHUSETTS
- (E) COUNTRY: USA
- (F) POSTAL CODE (ZIP): 01748
- (G) TELEPHONE: 1-508-435-9001
- (H) TELEFAX: 1-508-435-0454
- 15 (I) TELEX:

(ii) TITLE OF INVENTION: MORPHOGEN-INDUCED NERVE REGENERATION AND REPAIR

20 (iii) NUMBER OF SEQUENCES: 33

## (iv) CORRESPONDENCE ADDRESS:

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- 25 (C) CITY: HOPKINTON
- (D) STATE: MASSACHUSETTS
- (E) COUNTRY: USA
- (F) ZIP: 01748

## 30 (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 35 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## 35 (vi) ATTORNEY/AGENT INFORMATION:

- (A) NAME: KELLEY, ROBIN D.
- (B) REGISTRATION NUMBER: 34,637
- 40 (C) REFERENCE/DOCKET NUMBER: CRP-070

## 40 (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617/248-7000
- 45 (B) TELEFAX: 617/248-7100

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- 50 (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear



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(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- 5 (A) NAME/KEY: Protein  
(B) LOCATION: 1..97  
(D) OTHER INFORMATION: /label= GENERIC-SEQ1  
/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES  
10 ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER, A-AMINO  
ACIDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa  
20 20 25 30  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
25 35 40 45  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa  
50 55 60  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
65 70 75 80  
30 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys  
85 90 95  
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35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 97 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

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## (ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= GENERIC-SEQ2

/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES  
ONE OF THE 20 NATURALLY OCCURRING L-ISOMER A-AMINO  
ACIDS, OR A DERIVATIVE THEREOF."

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10 15  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa  
20 25 30  
Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
35 40 45  
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50 55 60  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
65 70 75 80  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys  
85 90 95  
Xaa

30

## (2) INFORMATION FOR SEQ ID NO:3:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

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(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= GENERIC-SEQ3

/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED  
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AS DEFINED IN THE SPECIFICATION."

50

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5 Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Ala  
 1 5 10 15  
 Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro  
 20 25 30  
 10 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Leu  
 35 40 45  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro  
 50 55 60  
 15 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 65 70 75 80  
 Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Gly Cys  
 85 90 95  
 20 Xaa

## (2) INFORMATION FOR SEQ ID NO:4:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 30 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

35

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= GENERIC-SEQ4  
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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa  
 1 5 10 15  
 Xaa Trp Xaa Xaa Ala Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly  
 20 25 30  
 50

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	Xaa	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	His	Ala
		35				40				45		
5	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
		50				55				60		
	Xaa	Cys	Cys	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Xaa
		65				70				75		80
10	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Met
					85					90		95
	Xaa	Xaa	Cys	Gly	Cys	Xaa						
				100								

(2) INFORMATION FOR SEQ ID NO:5:

**(1) SEQUENCE CHARACTERISTICS:**

- 20 (A) LENGTH: 139 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

**(v1) ORIGINAL SOURCE:**

- (A) ORGANISM: Homo sapiens  
(F) TISSUE TYPE: HIPPOCAMPUS

**(1x) FEATURE:**

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..139  
(D) OTHER INFORMATION: /label= hOP1-MATURE

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40	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	Ser
				20					25					30		
	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg
45			35					40					45			
	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala
	50						55					60				
50	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn
	65					70					75					80

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Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro  
                     85                    90                    95

5 Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile  
                     100                    105                    110

Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr  
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10 Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
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## (2) INFORMATION FOR SEQ ID NO:6:

## 15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE  
 (F) TISSUE TYPE: EMBRYO

25

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..139  
 (D) OTHER INFORMATION: /label= MOP1-MATURE

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     1                    5                    10                    15

Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser  
                     20                    25                    30

40 Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg  
                     35                    40                    45

45 Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala  
                     50                    55                    60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn  
                     65                    70                    75                    80

50 Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro  
                     85                    90                    95

- 114 -

Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile  
 100 105 110

5 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr  
 115 120 125

Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 130 135

10 (2) INFORMATION FOR SEQ ID NO:7:

(1) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 139 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS  
 (F) TISSUE TYPE: HIPPOCAMPUS

25 (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..139  
 (D) OTHER INFORMATION: /label= HOP2-MATURE

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu  
 1 5 10 15

35 Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser  
 20 25 30

His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln  
 35 40 45

40 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala  
 50 55 60

45 Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn  
 65 70 75 80

Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro  
 85 90 95

50 Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr  
 100 105 110

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Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His  
 115 120 125

Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
 130 135

5

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 139 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

20

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

## (ix) FEATURE:

25

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..139
- (D) OTHER INFORMATION: /label= MOP2-MATURE

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30

Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu  
 1 5 10 15

Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser  
 20 25 30

35

Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg  
 35 40 45

40

Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala  
 50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn  
 65 70 75 80

45

Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro  
 85 90 95

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Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr  
 100 105 110

5 Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His  
 115 120 125

Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
 130 135

10 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (vi) ORIGINAL SOURCE:

(A) ORGANISM: bovine

(ix) FEATURE:

- 25 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..101  
 (D) OTHER INFORMATION: /label= CBMP-2A-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

30 Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
 1 5 10 15

35 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly  
 20 25 30

Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
 35 40 45

40 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala  
 50 55 60

45 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
 65 70 75 80



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Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu  
85 90 95

Gly Cys Gly Cys Arg  
100

5

**(2) INFORMATION FOR SEQ ID NO:10:**

**(1) SEQUENCE CHARACTERISTICS:**

- SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 101 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: protein

15

**(vi) ORIGINAL SOURCE:**

- (A) ORGANISM: HOMO SAPIENS  
(F) TISSUE TYPE: hippocampus

20

**(ix) FEATURE:**

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..101  
(D) OTHER INFORMATION: /label= CBMP-2B-FX

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
1 5 10 15

Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly  
20 25 30

Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
35 40 45

Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala  
50 55 60

Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
65 70 75 80

Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu  
85 90 95

Gly Cys Gly Cys Arg  
100

45

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## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

10

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: DROSOPHILA MELANOGASTER

## (ix) FEATURE:

15

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..101  
 (D) OTHER INFORMATION: /label= DPP-FX

## 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp  
 1 5 10  
 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly  
 20 25 30  
 Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala  
 35 40 45  
 Val Val Gln Thr Leu Val Asn Asn Asn Pro Gly Lys Val Pro Lys  
 50 55 60  
 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu  
 65 70 75 80  
 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val  
 85 90 95  
 Val Gly Cys Gly Cys Arg  
 100

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50

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(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: XENOPUS

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGL-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15 Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln  
1 5 10 15  
Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly  
20 20 25 30  
Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala  
35 40 45  
Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu  
50 55 60  
25 Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr  
65 70 75 80  
30 Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val  
85 90 95  
Asp Glu Cys Gly Cys Arg  
100

35 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGR-1-FX

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5 Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln  
 1 5 10 15  
 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly  
 20 25 30  
 10 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys  
 50 55 60  
 15 Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe  
 65 70 75 80  
 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
 85 90 95  
 20 Arg Ala Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:14:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 30 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: protein  
 (iii) HYPOTHETICAL: NO  
 35 (iv) ANTI-SENSE: NO  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens  
 40 (F) TISSUE TYPE: brain  
 (ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..106  
 45 (D) OTHER INFORMATION: /note= "GDF-1 (fx)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

50 Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His  
 1 5 10 15

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Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly  
                     20                    25                    30  
 5 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala  
                     35                    40                    45  
 Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly  
                     50                    55                    60  
 10 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser  
                     65                    70                    75                    80  
 Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu  
                     85                    90                    95  
 15 Asp Met Val Val Asp Glu Cys Gly Cys Arg  
                     100                    105

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Xaa Xaa Xaa Xaa  
 1                    5

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1822 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: HOMO SAPIENS  
 (F) TISSUE TYPE: HIPPOCAMPUS

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## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 49..1341  
 (C) IDENTIFICATION METHOD: experimental  
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "OP1"  
 /evidence= EXPERIMENTAL  
 /standard\_name= "OP1"

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

5	GGT	GCG	GCG	CGG	AGC	CGG	CGG	GTA	GCG	CGT	AG	ATG	CAC	GTG	57
												Met	His	Val	
15												1			
	CGC	TCA	CTG	CGA	GCT	GCG	GCG	CCG	CAC	AGC	TTC	GTG	GCG	CTC	105
	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	Leu	
	5							10				15			
20	CCC	CTG	TTC	CTG	CTG	CGC	TCC	GCC	CTG	GCC	GAC	TTC	AGC	CTG	153
	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser	Leu	
	20					25					30			35	
25	GAG	GTG	CAC	TCG	AGC	TTC	ATC	CAC	CGG	CGC	CTC	CGC	AGC	CAG	201
	Glu	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	Gln	
					40					45				50	
30	CGG	GAG	ATG	CAG	CGC	GAG	ATC	CTC	TCC	ATT	TTG	GGC	TTG	CCC	249
	Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	Pro	
				55					60					65	
35	CCG	CGC	CCG	CAC	CTC	CAG	GGC	AAG	CAC	AAC	TCG	GCA	CCC	ATG	297
	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	
			70					75					80		
40	CTG	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	GGC	GGC	GGG	345
	Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	Gly	
	85						90					95			
45	GGC	CAG	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	393
	Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	
	100					105					110			115	
50	CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	441
	Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	
				120					125					130	
	ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	489
	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	
				135					140					145	

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	CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
			150					155					160				
5	CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
		165					170					175					
10	TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
	180					185					190					195	
15	CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
					200					205					210		
20	GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
	Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
				215					220					225			
25	ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
			230					235					240				
30	GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
		245					250					255					
35	AAG	TTG	GCG	GGC	CTG	ATT	GGG	CGG	CAC	GGG	CCC	CAG	AAC	AAG	CAG	CCC	873
	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	
	260					265					270					275	
40	TTC	ATG	GTG	GCT	TTC	TTC	AAG	GCC	ACG	GAG	GTC	CAC	TTC	CGC	AGC	ATC	921
	Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe	Arg	Ser	Ile	
					280					285					290		
45	CGG	TCC	ACG	GGG	AGC	AAA	CAG	CGC	AGC	CAG	AAC	CGC	TCC	AAG	ACG	CCC	969
	Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	
				295				300					305				
50	AAG	AAC	CAG	GAA	GCC	CTG	CGG	ATG	GCC	AAC	GTG	GCA	GAG	AAC	AGC	AGC	1017
	Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	
			310					315					320				
55	AGC	GAC	CAG	AGG	CAG	GCC	TGT	AAG	AAG	CAC	GAG	CTG	TAT	GTC	AGC	TTC	1065
	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	
		325					330					335					
60	CGA	GAC	CTG	GGC	TGG	CAG	GAC	TGG	ATC	ATC	GCG	CCT	GAA	GGC	TAC	GCC	1113
	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	
	340					345					350					355	

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GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG 1161  
 Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met  
 360 365 370

5 AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC 1209  
 Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn  
 375 380 385

10 CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC 1257  
 Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala  
 390 395 400

15 ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA 1305  
 Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys  
 405 410 415

20 TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC 1351  
 Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG 1411  
 GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG 1471

25 TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC 1531  
 ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591

30 GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651  
 CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711  
 GGCGTGGCAA GGGGTGGGCA CATTGGTGTG TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771

35 CTGTAATAAA TGTACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A 1822

## (2) INFORMATION FOR SEQ ID NO:17:

40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 431 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

50 Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1 5 10 15



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Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 20 25 30  
 5 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35 40 45  
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50 55 60  
 10 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro  
 65 70 75 80  
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly  
 85 90 95  
 15 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser  
 100 105 110  
 Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr  
 115 120 125  
 20 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys  
 130 135 140  
 25 Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu  
 145 150 155 160  
 Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile  
 165 170 175  
 30 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile  
 180 185 190  
 Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu  
 195 200 205  
 35 Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu  
 210 215 220  
 40 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg  
 225 230 235 240  
 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser  
 245 250 255  
 45 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn  
 260 265 270  
 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe  
 275 280 285  
 50

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Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser  
 290 295 300  
 5 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu  
 305 310 315 320  
 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr  
 325 330 335  
 10 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu  
 340 345 350  
 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn  
 355 360 365  
 15 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His  
 370 375 380  
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln  
 20 385 390 395 400  
 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile  
 405 410 415  
 25 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

## (2) INFORMATION FOR SEQ ID NO:18:

- 30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1873 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 35 (ii) MOLECULE TYPE: cDNA  
 (iii) HYPOTHETICAL: NO  
 40 (iv) ANTI-SENSE: NO  
 (v) ORIGINAL SOURCE:  
 (A) ORGANISM: MURIDAE  
 (F) TISSUE TYPE: EMBRYO  
 45 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 104..1393  
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 50 /product= "HOP1"  
 /note= "HOP1 (CDNA)"

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## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:18:

	CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG	60
5	CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC Met His Val Arg 1	115
10	TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro 5 10 15 20	163
15	CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu 25 30 35	211
20	GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg 40 45 50	259
25	GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro 55 60 65	307
30	CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu 70 75 80	355
35	GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln 85 90 95 100	403
40	GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro 105 110 115	451
45	TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val 120 125 130	499
50	ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro 135 140 145	547
55	CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu 150 155 160	595
60	GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile 165 170 175 180	643

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	CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
				185						190					195		
5	CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
	Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
				200					205					210			
10	CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
	Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
			215					220					225				
15	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
			230				235					240					
20	CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
						250					255					260	
25	GCA	GGC	CTG	ATT	GGA	CGG	CAT	GGA	CCC	CAG	AAC	AAG	CAA	CCC	TTC	ATG	931
	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	Phe	Met	
					265					270					275		
30	GTG	GCC	TTC	TTC	AAG	GCC	ACG	GAA	GTC	CAT	CTC	CGT	AGT	ATC	CGG	TCC	979
	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Leu	Arg	Ser	Ile	Arg	Ser	
					280				285					290			
35	ACG	GGG	GGC	AAG	CAG	CGC	AGC	CAG	AAT	CGC	TCC	AAG	ACG	CCA	AAG	AAC	1027
	Thr	Gly	Gly	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	
				295				300					305				
40	CAA	GAG	GCC	CTG	AGG	ATG	GCC	AGT	GTG	GCA	GAA	AAC	AGC	AGC	AGT	GAC	1075
	Gln	Glu	Ala	Leu	Arg	Met	Ala	Ser	Val	Ala	Glu	Asn	Ser	Ser	Ser	Asp	
			310				315					320					
45	CAG	AGG	CAG	GCC	TGC	AAG	AAA	CAT	GAG	CTG	TAC	GTC	AGC	TTC	CGA	GAC	1123
	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	
						330					335					340	
50	CTT	GGC	TGG	CAG	GAC	TGG	ATC	ATT	GCA	CCT	GAA	GGC	TAT	GCT	GCC	TAC	1171
	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala	Tyr	
					345					350					355		
55	TAC	TGT	GAG	GGA	GAG	TGC	GCC	TTC	CCT	CTG	AAC	TCC	TAC	ATG	AAC	GCC	1219
	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala	
					360				365					370			
60	ACC	AAC	CAC	GCC	ATC	GTC	CAG	ACA	CTG	GTT	CAC	TTC	ATC	AAC	CCA	GAC	1267
	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe	Ile	Asn	Pro	Asp	
				375				380					385				

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ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT 1315  
 Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser  
 390 395 400

5 GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC GAC CTG AAG AAG TAC AGA 1363  
 Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu Lys Lys Tyr Arg  
 405 410 415 420

AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCCTG 1413  
 10 Asn Met Val Val Arg Ala Cys Gly Cys His  
 425 430

ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG 1473

15 CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG 1533

AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCTTCT 1593

GGCAGGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT 1653  
 20 GTCTGCCAGG AAAGTGTCCT GTGTCCACAT GGCCCTGGC GCTCTGAGTC TTGAGGAGT 1713

AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCGCTGGCG 1773

25 TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT 1833

GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC 1873

## 30 (2) INFORMATION FOR SEQ ID NO:19:

## (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids  
 (B) TYPE: amino acid  
 35 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

40 Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1 5 10 15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 45 20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35 40 45

50 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50 55 60

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Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro  
 65 70 75 80  
 5 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly  
 85 90 95  
 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr  
 100 105 110  
 10 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp  
 115 120 125  
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu  
 130 135 140  
 15 Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser  
 145 150 155 160  
 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr  
 165 170 175  
 20 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr  
 180 185 190  
 25 Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe  
 195 200 205  
 Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val  
 210 215 220  
 30 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His  
 225 230 235 240  
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile  
 245 250 255  
 35 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys  
 260 265 270  
 40 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg  
 275 280 285  
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys  
 290 295 300  
 45 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn  
 305 310 315 320  
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val  
 325 330 335  
 50

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	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly
				340					345						350	
5	Tyr	Ala	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser
			355					360					365			
	Tyr	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe
		370					375					380				
10	Ile	Asn	Pro	Asp	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	Leu
	385					390					395					400
	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Ser	Ser	Asn	Val	Asp	Leu
					405					410					415	
15	Lys	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	His		
				420					425					430		

**(2) INFORMATION FOR SEQ ID NO:20:**

**(1) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 1723 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

**(ii) MOLECULE TYPE: cDNA**

**(v1) ORIGINAL SOURCE:**

- (A) ORGANISM: Homo sapiens  
(F) TISSUE TYPE: HIPPOCAMPUS

**(ix) FEATURE:**

- (A) NAME/KEY: CDS  
(B) LOCATION: 490..1696  
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
/product= "hOP2-PP"  
/note= "hOP2 (cDNA)"

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA	60
45	GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCCAGG AGGCGCTGGA GCAACAGCTC	120
	CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCTGCGC TGCTCGGACC	180
50	GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT	240

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	CCGCAGAGTA GCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG	300
	GACAGGTGTC GCGCGGCGGG GCTCCAGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC	360
5	CGCCCCGCCC CGCCGCCGC CGCCGCGA GCCAGCCTC CTGCGGTCG GGGCGTCCCC	420
	AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCGCTGA GCGCCCCAGC TGAGCGCCCC	480
10	CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	528
	1 5 10	
15	GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro	576
	15 20 25	
20	GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	624
	30 35 40 45	
25	CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	672
	50 55 60	
30	GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	720
	65 70 75	
35	CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	768
	80 85 90	
40	CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val	816
	95 100 105	
45	AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp	864
	110 115 120 125	
50	AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val	912
	130 135 140	
55	ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu	960
	145 150 155	
60	AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser	1008
	160 165 170	



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	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	CTC	CGA	GCT	1056
	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ala	
	175						180					185					
5	GGA	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAT	GTC	ACA	GCA	GCC	AGT	GAC	TGC	1104
	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Val	Thr	Ala	Ala	Ser	Asp	Cys	
	190					195					200					205	
10	TGG	TTG	CTG	AAG	CGT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	TAT	GTG	GAG	1152
	Trp	Leu	Leu	Lys	Arg	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	
					210					215					220		
15	ACT	GAG	GAC	GGG	CAC	AGC	GTG	GAT	CCT	GGC	CTG	GCC	GGC	CTG	CTG	GGT	1200
	Thr	Glu	Asp	Gly	His	Ser	Val	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	
				225					230					235			
20	CAA	CGG	GCC	CCA	CGC	TCC	CAA	CAG	CCT	TTC	GTG	GTC	ACT	TTC	TTC	AGG	1248
	Gln	Arg	Ala	Pro	Arg	Ser	Gln	Gln	Pro	Phe	Val	Val	Thr	Phe	Phe	Arg	
			240					245					250				
25	GCC	AGT	CCG	AGT	CCC	ATC	CGC	ACC	CCT	CGG	GCA	GTG	AGG	CCA	CTG	AGG	1296
	Ala	Ser	Pro	Ser	Pro	Ile	Arg	Thr	Pro	Arg	Ala	Val	Arg	Pro	Leu	Arg	
			255				260					265					
30	AGG	AGG	CAG	CCG	AAG	AAA	AGC	AAC	GAG	CTG	CCG	CAG	GCC	AAC	CGA	CTC	1344
	Arg	Arg	Gln	Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln	Ala	Asn	Arg	Leu	
						275					280					285	
35	CCA	GGG	ATC	TTT	GAT	GAC	GTC	CAC	GGC	TCC	CAC	GGC	CGG	CAG	GTC	TGC	1392
	Pro	Gly	Ile	Phe	Asp	Asp	Val	His	Gly	Ser	His	Gly	Arg	Gln	Val	Cys	
					290					295					300		
40	CGT	CGG	CAC	GAG	CTC	TAC	GTC	AGC	TTC	CAG	GAC	CTC	GGC	TGG	CTG	GAC	1440
	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp	
				305					310					315			
45	TGG	GTC	ATC	GCT	CCC	CAA	GGC	TAC	TCG	GCC	TAT	TAC	TGT	GAG	GGG	GAG	1488
	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	
			320				325						330				
50	TGC	TCC	TTC	CCA	CTG	GAC	TCC	TGC	ATG	AAT	GCC	ACC	AAC	CAC	GCC	ATC	1536
	Cys	Ser	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	
			335				340					345					
55	CTG	CAG	TCC	CTG	GTG	CAC	CTG	ATG	AAG	CCA	AAC	GCA	GTC	CCC	AAG	GCG	1584
	Leu	Gln	Ser	Leu	Val	His	Leu	Met	Lys	Pro	Asn	Ala	Val	Pro	Lys	Ala	
						355					360					365	

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TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC 1632  
 Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp  
 370 375 380  
 5 AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC AAG 1680  
 Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys  
 385 390 395  
 GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG 1723  
 10 Ala Cys Gly Cys His  
 400

## (2) INFORMATION FOR SEQ ID NO:21:

15

- (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 402 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

20

(11) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:21:

25 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys  
 1 5 10 15  
 Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro  
 20 25 30  
 30 Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile  
 35 40 45  
 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro  
 35 50 55 60  
 Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu  
 65 70 75 80  
 40 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu  
 85 90 95  
 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val  
 100 105 110  
 45 Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe  
 115 120 125  
 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala  
 50 130 135 140

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Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr  
 145 150 155 160  
 5 Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu  
 165 170 175  
 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu  
 180 185 190  
 10 Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu  
 195 200 205  
 Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp  
 210 215 220  
 15 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala  
 225 230 235 240  
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro  
 245 250 255  
 20 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln  
 260 265 270  
 25 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile  
 275 280 285  
 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His  
 290 295 300  
 30 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile  
 305 310 315 320  
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe  
 325 330 335  
 35 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser  
 340 345 350  
 40 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala  
 355 360 365  
 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn  
 370 375 380  
 45 Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly  
 385 390 395 400  
 Cys His  
 50

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## (2) INFORMATION FOR SEQ ID NO:22:

## (1) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1926 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (vi) ORIGINAL SOURCE:

- 10 (A) ORGANISM: MURIDAE  
 (F) TISSUE TYPE: EMBRYO

## (ix) FEATURE:

- 15 (A) NAME/KEY: CDS  
 (B) LOCATION: 93..1289  
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
 /product= "mOP2-PP"  
 /note= "mOP2 cDNA"

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT 60

25 ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA 113  
 Met Ala Met Arg Pro Gly Pro  
 1 5

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT 161  
 30 Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly  
 10 15 20

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG 209  
 35 Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu  
 25 30 35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA 257  
 Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly  
 40 45 50 55

CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC 305  
 Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser  
 60 65 70

GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC 353  
 45 Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp  
 75 80 85

GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG 401  
 50 Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met  
 90 95 100

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	AGC	TTC	GTC	AAC	ATG	GTG	GAA	CGC	GAC	CGT	ACC	CTG	GGC	TAC	CAG	GAG	449
	Ser	Phe	Val	Asn	Met	Val	Glu	Arg	Asp	Arg	Thr	Leu	Gly	Tyr	Gln	Glu	
	105						110					115					
5	CCA	CAC	TGG	AAG	GAA	TTC	CAC	TTT	GAC	CTA	ACC	CAG	ATC	CCT	GCT	GGG	497
	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp	Leu	Thr	Gln	Ile	Pro	Ala	Gly	
	120				125					130						135	
10	GAG	GCT	GTC	ACA	GCT	GCT	GAG	TTC	CGG	ATC	TAC	AAA	GAA	CCC	AGC	ACC	545
	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	
				140					145						150		
15	CAC	CCG	CTC	AAC	ACA	ACC	CTC	CAC	ATC	AGC	ATG	TTC	GAA	GTG	GTC	CAA	593
	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile	Ser	Met	Phe	Glu	Val	Val	Gln	
				155					160					165			
	GAG	CAC	TCC	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	641
	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	
			170					175					180				
20	CTC	CGA	TCT	GGG	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAC	ATC	ACA	GCA	GCC	689
	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Ile	Thr	Ala	Ala	
		185					190					195					
25	AGT	GAC	CGA	TGG	CTG	CTG	AAC	CAT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	737
	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	
	200				205					210						215	
	TAT	GTG	GAA	ACC	GCG	GAT	GGG	CAC	AGC	ATG	GAT	CCT	GGC	CTG	GCT	GGT	785
30	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	Met	Asp	Pro	Gly	Leu	Ala	Gly	
				220						225					230		
	CTG	CTT	GGA	CGA	CAA	GCA	CCA	CGC	TCC	AGA	CAG	CCT	TTC	ATG	GTA	ACC	833
35	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	Arg	Gln	Pro	Phe	Met	Val	Thr	
			235					240					245				
	TTC	TTC	AGG	GCC	AGC	CAG	AGT	CCT	GTG	CGG	GCC	CCT	CGG	GCA	GCG	AGA	881
	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	Arg	Ala	Pro	Arg	Ala	Ala	Arg	
			250					255					260				
40	CCA	CTG	AAG	AGG	AGG	CAG	CCA	AAG	AAA	ACG	AAC	GAG	CTT	CCG	CAC	CCC	929
	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His	Pro	
		265					270					275					
45	AAC	AAA	CTC	CCA	GGG	ATC	TTT	GAT	GAT	GGC	CAC	GGT	TCC	CGC	GGC	AGA	977
	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	
		280				285				290						295	
50	GAG	GTT	TGC	CGC	AGG	CAT	GAG	CTC	TAC	GTC	AGC	TTC	CGT	GAC	CTT	GGC	1025
	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	
				300						305					310		

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	TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT	1073
	Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys	
	315 320 325	
5	GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC	1121
	Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn	
	330 335 340	
10	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
	His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
	345 350 355	
15	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
	Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
	360 365 370 375	
20	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
	Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
	380 385 390	
20	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCGG CCCAGCATCC TGCTTCTACT	1319
	Val Val Lys Ala Cys Gly Cys His	
	395	
25	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
	CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA AAATTCTGGT	1439
	CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
30	TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
	CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC	1619
35	AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT	1679
	CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTITAGGT ATAACAGACA CATACTTA	1739
	GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
40	CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859
	CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAAC	1919
45	GGAATTC	1926

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## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 399 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys  
1 5 10 15

15 Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln  
20 25 30

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu  
35 40 45

20 Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala  
50 55 60

25 Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr  
65 70 75 80

His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu  
85 90 95

30 Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp  
100 105 110

Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp  
115 120 125

35 Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg  
130 135 140

40 Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile  
145 150 155 160

Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu  
165 170 175

45 Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu  
180 185 190

Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His  
195 200 205

50 Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser  
210 215 220

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Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser  
 225 230 235 240

5 Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val  
 245 250 255

Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys  
 260 265 270

10 Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp  
 275 280 285

Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr  
 290 295 300

15 Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln  
 305 310 315 320

Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp  
 20 325 330 335

Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His  
 340 345 350

25 Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys  
 355 360 365

Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile  
 370 375 380

30 Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
 385 390 395

## (2) INFORMATION FOR SEQ ID NO:24:

35

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1368 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

## (ii) MOLECULE TYPE: cDNA

45

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1368

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:



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	ATG	TCG	GGA	CTG	CGA	AAC	ACC	TCG	GAG	GCC	GTT	GCA	GTG	CTC	GCC	TCC	48
	Met	Ser	Gly	Leu	Arg	Asn	Thr	Ser	Glu	Ala	Val	Ala	Val	Leu	Ala	Ser	
	1				5				10					15			
5	CTG	GGA	CTC	GGA	ATG	GTT	CTG	CTC	ATG	TTC	GTG	GCG	ACC	ACG	CCG	CCG	96
	Leu	Gly	Leu	Gly	Met	Val	Leu	Leu	Met	Phe	Val	Ala	Thr	Thr	Pro	Pro	
				20					25					30			
10	GCC	GTT	GAG	GCC	ACC	CAG	TCG	GGG	ATT	TAC	ATA	GAC	AAC	GGC	AAG	GAC	144
	Ala	Val	Glu	Ala	Thr	Gln	Ser	Gly	Ile	Tyr	Ile	Asp	Asn	Gly	Lys	Asp	
			35					40					45				
15	CAG	ACG	ATC	ATG	CAC	AGA	GTG	CTG	AGC	GAG	GAC	GAC	AAG	CTG	GAC	GTC	192
	Gln	Thr	Ile	Met	His	Arg	Val	Leu	Ser	Glu	Asp	Asp	Lys	Leu	Asp	Val	
		50					55					60					
20	TCG	TAC	GAG	ATC	CTC	GAG	TTC	CTG	GGC	ATC	GCC	GAA	CGG	CCG	ACG	CAC	240
	Ser	Tyr	Glu	Ile	Leu	Glu	Phe	Leu	Gly	Ile	Ala	Glu	Arg	Pro	Thr	His	
	65					70				75						80	
25	CTG	AGC	AGC	CAC	CAG	TTG	TCG	CTG	AGG	AAG	TCG	GCT	CCC	AAG	TTC	CTG	288
	Leu	Ser	Ser	His	Gln	Leu	Ser	Leu	Arg	Lys	Ser	Ala	Pro	Lys	Phe	Leu	
				85						90					95		
30	GAT	GAG	GAC	GAC	GAC	TAC	GAA	CGC	GGC	CAT	CGG	TCC	AGG	AGG	AGC	GCC	384
	Asp	Glu	Asp	Asp	Asp	Tyr	Glu	Arg	Gly	His	Arg	Ser	Arg	Arg	Ser	Ala	
			115					120					125				
35	GAC	CTC	GAG	GAG	GAT	GAG	GGC	GAG	CAG	CAG	AAG	AAC	TTC	ATC	ACC	GAC	432
	Asp	Leu	Glu	Glu	Asp	Glu	Gly	Glu	Gln	Gln	Lys	Asn	Phe	Ile	Thr	Asp	
		130					135					140					
40	CTG	GAC	AAG	CGG	GCC	ATC	GAC	GAG	AGC	GAC	ATC	ATC	ATG	ACC	TTC	CTG	480
	Leu	Asp	Lys	Arg	Ala	Ile	Asp	Glu	Ser	Asp	Ile	Ile	Met	Thr	Phe	Leu	
	145					150					155					160	
45	AAC	AAG	CGC	CAC	CAC	AAT	GTG	GAC	GAA	CTG	CGT	CAC	GAG	CAC	GGC	CGT	528
	Asn	Lys	Arg	His	His	Asn	Val	Asp	Glu	Leu	Arg	His	Glu	His	Gly	Arg	
				165						170					175		
50	CGC	CTG	TGG	TTC	GAC	GTC	TCC	AAC	GTG	CCC	AAC	GAC	AAC	TAC	CTG	GTG	576
	Arg	Leu	Trp	Phe	Asp	Val	Ser	Asn	Val	Pro	Asn	Asp	Asn	Tyr	Leu	Val	
				180					185					190			
55	ATG	GCC	GAG	CTG	CGC	ATC	TAT	CAG	AAC	GCC	AAC	GAG	GGC	AAG	TGG	CTG	624
	Met	Ala	Glu	Leu	Arg	Ile	Tyr	Gln	Asn	Ala	Asn	Glu	Gly	Lys	Trp	Leu	
			195					200					205				

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	ACC	GCC	AAC	AGG	GAG	TTC	ACC	ATC	ACG	GTA	TAC	GCC	ATT	GGC	ACC	GGC	672
	Thr	Ala	Asn	Arg	Glu	Phe	Thr	Ile	Thr	Val	Tyr	Ala	Ile	Gly	Thr	Gly	
	210						215					220					
5	ACG	CTG	GGC	CAG	CAC	ACC	ATG	GAG	CCG	CTG	TCC	TCG	GTG	AAC	ACC	ACC	720
	Thr	Leu	Gly	Gln	His	Thr	Met	Glu	Pro	Leu	Ser	Ser	Val	Asn	Thr	Thr	
	225					230					235					240	
10	GGG	GAC	TAC	GTG	GGC	TGG	TTG	GAG	CTC	AAC	GTG	ACC	GAG	GGC	CTG	CAC	768
	Gly	Asp	Tyr	Val	Gly	Trp	Leu	Glu	Leu	Asn	Val	Thr	Glu	Gly	Leu	His	
				245						250					255		
15	GAG	TGG	CTG	GTC	AAG	TCG	AAG	GAC	AAT	CAT	GGC	ATC	TAC	ATT	GGA	GCA	816
	Glu	Trp	Leu	Val	Lys	Ser	Lys	Asp	Asn	His	Gly	Ile	Tyr	Ile	Gly	Ala	
				260					265					270			
20	CAC	GCT	GTC	AAC	CGA	CCC	GAC	CGC	GAG	GTG	AAG	CTG	GAC	GAC	ATT	GGA	864
	His	Ala	Val	Asn	Arg	Pro	Asp	Arg	Glu	Val	Lys	Leu	Asp	Asp	Ile	Gly	
			275					280					285				
	CTG	ATC	CAC	CGC	AAG	GTG	GAC	GAC	GAG	TTC	CAG	CCC	TTC	ATG	ATC	GGC	912
	Leu	Ile	His	Arg	Lys	Val	Asp	Asp	Glu	Phe	Gln	Pro	Phe	Met	Ile	Gly	
	290						295					300					
25	TTC	TTC	CGC	GGA	CCG	GAG	CTG	ATC	AAG	GCG	ACG	GCC	CAC	AGC	AGC	CAC	960
	Phe	Phe	Arg	Gly	Pro	Glu	Leu	Ile	Lys	Ala	Thr	Ala	His	Ser	Ser	His	
	305					310					315					320	
30	CAC	AGG	AGC	AAG	CGA	AGC	GCC	AGC	CAT	CCA	CGC	AAG	CGC	AAG	AAG	TCG	1008
	His	Arg	Ser	Lys	Arg	Ser	Ala	Ser	His	Pro	Arg	Lys	Arg	Lys	Lys	Ser	
				325						330					335		
35	GTG	TCG	CCC	AAC	AAC	GTG	CCG	CTG	CTG	GAA	CCG	ATG	GAG	AGC	ACG	CGC	1056
	Val	Ser	Pro	Asn	Asn	Val	Pro	Leu	Leu	Glu	Pro	Met	Glu	Ser	Thr	Arg	
				340					345					350			
40	AGC	TGC	CAG	ATG	CAG	ACC	CTG	TAC	ATA	GAC	TTC	AAG	GAT	CTG	GGC	TGG	1104
	Ser	Cys	Gln	Met	Gln	Thr	Leu	Tyr	Ile	Asp	Phe	Lys	Asp	Leu	Gly	Trp	
			355					360					365				
	CAT	GAC	TGG	ATC	ATC	GCA	CCA	GAG	GGC	TAT	GGC	GCC	TTC	TAC	TGC	AGC	1152
	His	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Gly	Ala	Phe	Tyr	Cys	Ser	
	370						375					380					
45	GGC	GAG	TGC	AAT	TTC	CCG	CTC	AAT	GCG	CAC	ATG	AAC	GCC	ACG	AAC	CAT	1200
	Gly	Glu	Cys	Asn	Phe	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	
	385					390					395					400	
50	GCG	ATC	GTC	CAG	ACC	CTG	GTC	CAC	CTG	CTG	GAG	CCC	AAG	AAG	GTG	CCC	1248
	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Leu	Leu	Glu	Pro	Lys	Lys	Val	Pro	
				405						410					415		

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AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC 1296  
 Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr  
                     420                    425                    430

5 CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT 1344  
 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile  
                     435                    440                    445

10 GTG AAA TCC TGC GGG TGC CAT TGA 1368  
 Val Lys Ser Cys Gly Cys His  
                     450                    455

## (2) INFORMATION FOR SEQ ID NO:25:

15

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 455 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

25 Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser  
       1                    5                    10                    15

Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro  
                     20                    25                    30

30 Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp  
                     35                    40                    45

35 Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val  
       50                    55                    60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His  
       65                    70                    75                    80

40 Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu  
                     85                    90                    95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln  
                     100                    105                    110

45 Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala  
                     115                    120                    125

50 Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp  
       130                    135                    140

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Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu  
 145 150 155 160  
 5 Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg  
 165 170 175  
 Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val  
 180 185 190  
 10 Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu  
 195 200 205  
 Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly  
 210 215 220  
 15 Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr  
 225 230 235 240  
 20 Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His  
 245 250 255  
 Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala  
 260 265 270  
 25 His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly  
 275 280 285  
 Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly  
 290 295 300  
 30 Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His  
 305 310 315 320  
 35 His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser  
 325 330 335  
 Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg  
 340 345 350  
 40 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp  
 355 360 365  
 His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser  
 370 375 380  
 45 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
 385 390 395 400  
 50 Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro  
 405 410 415

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Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr  
 420 425 430

5 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile  
 435 440 445

Val Lys Ser Cys Gly Cys His  
 450 455

10

## (2) INFORMATION FOR SEQ ID NO:26:

## (1) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 104 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- 25 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..104  
 (D) OTHER INFORMATION: /note= "BMP3"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

30 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser  
 1 5 10 15

Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly  
 20 25 30

35

Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala  
 35 40 45

40

Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile  
 50 55 60

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu  
 65 70 75 80

45

Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met  
 85 90 95

Thr Val Glu Ser Cys Ala Cys Arg  
 100

50

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## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

10

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

## (ix) FEATURE:

15

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /note= "BMP5"

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
 1 5 10 15

25

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly  
 20 25 30

30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 35 40 45

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys  
 50 55 60

35

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
 65 70 75 80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
 85 90 95

40

Arg Ser Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:28:

45

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

50

## (ii) MOLECULE TYPE: protein

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(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /note= "BMP6"

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln  
 1 5 10 15  
 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly  
 20 25 30  
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys  
 50 55 60  
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
 65 70 75 80  
 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val  
 85 90 95  
 Arg Ala Cys Gly Cys His  
 100

(2) INFORMATION FOR SEQ ID NO:29:

35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= OPX  
 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED  
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS  
 AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

50

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

5 Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa  
 1 5 10 15  
 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly  
 20 25 30  
 10 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys  
 50 55 60  
 15 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa  
 65 70 75 80  
 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val  
 85 90 95  
 20 Xaa Ala Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:30:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 97 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 30 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (ix) FEATURE:

35 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..97  
 (D) OTHER INFORMATION: /label= GENERIC-SEQ5  
 40 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED  
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS  
 AS DEFINED IN THE SPECIFICATION."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

45 Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Xaa  
 1 5 10 15  
 Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro  
 20 25 30  
 50



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[illegible]

15

**(2) INFORMATION FOR SEQ ID NO:31:**

**(1) SEQUENCE CHARACTERISTICS:**

- 20 (A) LENGTH: 102 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

25

**(ix) FEATURE:**

- 30 (A) NAME/KEY: Protein  
(B) LOCATION: 1..102  
(D) OTHER INFORMATION: /label= GENERIC-SEQ6  
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED  
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS  
AS DEFINED IN THE SPECIFICATION. "

35

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:31:

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	Xaa	Gly	Trp	Xaa
	1				5					10					15	
40	Xaa	Trp	Xaa	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Tyr	Cys	Xaa	Gly
				20					25					30		
	Xaa	Cys	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	His	Ala
45			35					40					45			
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
		50					55					60				
50	Xaa	Cys	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa
	65					70					75					80

- 150 -

Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val  
85 90 95

5 Xaa Xaa Cys Xaa Cys Xaa 100

**(2) INFORMATION FOR SEQ ID NO:32:**

10 (4) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1247 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (11) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: HOMO SAPIENS  
(F) TISSUE TYPE: BRAIN

(1x) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 84..1199  
 (D) OTHER INFORMATION: /product= "GDF-1"  
 /note= "GDF-1 CDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

30	GGGGACACCG	GCCCCGCCCT	CAGCCCACTG	GTCCCGGGCC	GCCGCGGACC	CTGCGCACTC	60
	TCTGGTCATC	GCCTGGGAGG	AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC	110			
			Met Pro Pro Pro Gln Gln Gly Pro Cys				
			1 5				
35	GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC	158					
	Gly His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro						
	10 15 20 25						
40	CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG	206					
	Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln						
	30 35 40						
	GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG	254					
45	Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro						
	45 50 55						
	GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG	302					
	Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu						
50	60 65 70						

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	ACC	AGG	TCT	GGC	TCG	CGG	CGG	ACG	TCC	CCA	GGG	GTC	ACC	CTG	CAA	CCG	350
	Thr	Arg	Ser	Gly	Ser	Arg	Arg	Thr	Ser	Pro	Gly	Val	Thr	Leu	Gln	Pro	
	75						80					85					
5	TGC	CAC	GTG	GAG	GAG	CTG	GGG	GTC	GCC	GGA	AAC	ATC	GTG	CGC	CAC	ATC	398
	Cys	His	Val	Glu	Glu	Leu	Gly	Val	Ala	Gly	Asn	Ile	Val	Arg	His	Ile	
	90					95					100					105	
10	CCG	GAC	CGC	GGT	GCG	CCC	ACC	CGG	GCC	TCG	GAG	CCT	GTC	TCG	GCC	GCG	446
	Pro	Asp	Arg	Gly	Ala	Pro	Thr	Arg	Ala	Ser	Glu	Pro	Val	Ser	Ala	Ala	
					110					115					120		
15	GGG	CAT	TGC	CCT	GAG	TGG	ACA	GTC	GTC	TTC	GAC	CTG	TCG	GCT	GTG	GAA	494
	Gly	His	Cys	Pro	Glu	Trp	Thr	Val	Val	Phe	Asp	Leu	Ser	Ala	Val	Glu	
				125				130						135			
20	CCC	GCT	GAG	CGC	CCG	AGC	CGG	GCC	CGC	CTG	GAG	CTG	CGT	TTC	GCG	GCG	542
	Pro	Ala	Glu	Arg	Pro	Ser	Arg	Ala	Arg	Leu	Glu	Leu	Arg	Phe	Ala	Ala	
			140					145					150				
25	GCG	GCG	GCG	GCA	GCC	CCG	GAG	GCG	GCG	TGG	GAG	CTG	AGC	GTG	GCG	CAA	590
	Ala	Ala	Ala	Ala	Ala	Pro	Glu	Gly	Gly	Trp	Glu	Leu	Ser	Val	Ala	Gln	
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30	TTG	GTG	CCC	GCC	CTG	GGG	CCG	CCA	GTG	CGC	GCG	GAG	CTG	CTG	GGC	GCC	686
	Leu	Val	Pro	Ala	Leu	Gly	Pro	Pro	Val	Arg	Ala	Glu	Leu	Leu	Gly	Ala	
					190					195					200		
35	GCT	TGG	GCT	CGC	AAC	GCC	TCA	TGG	CCG	CGC	AGC	CTC	CGC	CTG	GCG	CTG	734
	Ala	Trp	Ala	Arg	Asn	Ala	Ser	Trp	Pro	Arg	Ser	Leu	Arg	Leu	Ala	Leu	
				205					210					215			
40	GCG	CTA	CGC	CCC	CGG	GCC	CCT	GCC	GCC	TGC	GCG	CGC	CTG	GCC	GAG	GCC	782
	Ala	Leu	Arg	Pro	Arg	Ala	Pro	Ala	Ala	Cys	Ala	Arg	Leu	Ala	Glu	Ala	
			220					225					230				
45	TCG	CTG	CTG	CTG	GTG	ACC	CTC	GAC	CCG	CGC	CTG	TGC	CAC	CCC	CTG	GCC	830
	Ser	Leu	Leu	Leu	Val	Thr	Leu	Asp	Pro	Arg	Leu	Cys	His	Pro	Leu	Ala	
							240					245					
50	CGG	CCG	CGG	CGC	GAC	GCC	GAA	CCC	GTG	TTG	GGC	GGC	GGC	CCC	GGG	GGC	878
	Arg	Pro	Arg	Arg	Asp	Ala	Glu	Pro	Val	Leu	Gly	Gly	Gly	Pro	Gly	Gly	
	250					255					260					265	
55	GCT	TGT	CGC	GCG	CGG	CTG	TAC	GTG	AGC	TTC	CGC	GAG	GTG	GGC	TGG		926
	Ala	Cys	Arg	Ala	Arg	Arg	Leu	Tyr	Val	Ser	Phe	Arg	Glu	Val	Gly	Trp	
					270					275					280		

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CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG 974  
 His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln  
 285 290 295

5 GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG 1022  
 Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro  
 300 305 310

10 GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG 1070  
 Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro  
 315 320 325

15 GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC 1118  
 Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile  
 330 335 340 345

20 TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT 1166  
 Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr  
 350 355 360

GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGA 1219  
 Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg  
 365 370

25 CCCGGGCCCA ACAATAAATG CCGCGTGG 1247

## (2) INFORMATION FOR SEQ ID NO:33:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 372 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

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 1 5 10 15

Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro  
 20 25 30

45 Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu  
 35 40 45

Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg  
 50 55 60

50 Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg  
 65 70 75 80

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Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly  
 85 90 95  
 5 Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr  
 100 105 110  
 Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr  
 115 120 125  
 10 Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg  
 130 135 140  
 Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu  
 145 150 155 160  
 15 Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala  
 165 170 175  
 Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro  
 180 185 190  
 20 Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser  
 195 200 205  
 25 Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro  
 210 215 220  
 Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu  
 225 230 235 240  
 30 Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu  
 245 250 255  
 35 Pro Val Leu Gly Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu  
 260 265 270  
 Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro  
 275 280 285  
 40 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val  
 290 295 300  
 Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu  
 305 310 315 320  
 45

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Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys  
325 330 335

5 Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn  
340 345 350

Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu  
355 360 365

10 Cys Gly Cys Arg  
370

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What is claimed is:

1. The use of a morphogen in the manufacture of a pharmaceutical for enhancing survival of neural cells at risk of dying.
2. A method for enhancing survival of neural cells at risk of dying, the method comprising providing a morphogen to said cells at a concentration and for a time sufficient to enhance survival of said cells.
3. The invention of claim 1 or 2 wherein said cells are at risk of dying due to chemical or mechanical trauma to nerve tissue comprising said cells.
4. The invention of claim 3 wherein said trauma comprises a transected nerve.
5. The invention of claim 3 wherein said morphogen is provided to said cells prior to said trauma.
6. The invention of claim 3 wherein said trauma results in demyelination of said cells.
7. The invention of claim 3 wherein said trauma results from exposure of said cells to a cellular toxin.
8. The invention of claim 7 wherein said toxin comprises ethanol.

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9. The invention of claim 1 or 2 wherein said cells are at risk of dying due to a neuropathy.
- 5 10. The invention of claim 9 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.
- 10 11. The invention of claim 10 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis or Alzheimer's disease.
- 15 12. The invention of claim 1 or 2 wherein said cells are at risk of dying due a neoplastic lesion associated with nerve tissue comprising said cells.
- 20 13. The invention of claim 12 wherein said lesion results from a neoplasm comprising cells of neuronal origin.
- 25 14. The invention of claim 13 wherein said neoplasm comprises a neuroblastoma or a retinoblastoma.
15. The invention of claim 12 wherein said lesion results from a neoplasm comprising glial cells.
- 30 16. The invention of claim 1 or 2 wherein said neural cells at risk of dying comprise part of the central nervous system.
17. The invention of claim 16 wherein said cells comprise striatal basal ganglia neurons.



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18. The invention of claim 16 wherein said cells  
comprise neurons of the substantia nigra.
- 5 19. The invention of claim 1 or 2 wherein said cells at  
risk of dying comprise part of the peripheral  
nervous system.
- 10 20. The invention of claim 1 or 2 wherein said  
morphogen stimulates cell adhesion molecule  
production in said cells.
- 15 21. The invention of claim 20 wherein said cell  
adhesion molecule is a nerve cell adhesion  
molecule.
22. The invention of claim 21 wherein nerve cell  
adhesion molecule is selected from the group  
consisting of N-CAM-120, N-CAM-140 and N-CAM-180.
- 20 23. The invention of claim 1 or 2 wherein said  
morphogen comprises an amino acid sequence sharing  
at least 70% homology with one of the sequences  
selected from the group consisting of: OP-1, OP-2,  
CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and  
25 60A(fx).
24. The invention of claim 23 wherein said morphogen  
comprises an amino acid sequence sharing at least  
80% homology with one of the sequences selected  
30 from the group consisting of: OP-1, OP-2, CBMP2,  
Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).

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- 5 25. The invention of claim 24 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
- 10 26. The invention of claim 25 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
- 15 27. The invention of claim 22 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
- 20 28. A method for enhancing the survival of neural cells at risk of dying in a mammal, the method comprising the step of administering to said mammal an effective amount of an agent capable of stimulating production of an endogenous morphogen.
- 25 29. The method of claim 28 wherein said agent stimulates production of an endogenous morphogen in the tissue comprising said neural cells.
- 30 30. A method for maintaining a neural pathway in a mammal, comprising:  
providing a morphogen to the neurons defining said pathway at a concentration and for a time sufficient to maintain said pathway.
31. The method of claim 30 wherein said morphogen is provided prior to injury to said pathway.

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32. The method of claim 30 wherein said morphogen is sufficient to stimulate repair of a damaged neural pathway.
- 5 33. The method of claim 32 wherein said damaged neural pathway results from mechanical or chemical trauma to said pathway.
34. The method of claim 33 wherein said trauma  
10 comprises a severed nerve.
35. The method of claim 33 wherein said trauma comprises demyelination of the neurons defining said pathway.  
15
36. The method of claim 33 wherein said trauma results from exposure of the cells defining said pathway to a cellular toxin.
- 20 37. The method of claim 36 wherein said toxin comprises ethanol.
38. The method of claim 30 wherein said damaged neural pathway results from a neuropathy of the cells  
25 defining said pathway.
39. The method of claim 38 wherein the etiology of said neuropathy is metabolic, infectious, toxic, autoimmune, nutritional, or ischemic.  
30
40. The method of claim 39 wherein said neuropathy comprises Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, or Alzheimer's disease.  
35

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41. The method of claim 38 wherein said neuropathy comprises axonal degeneration.
- 5 42. The method of claim 38 wherein said neuropathy comprises a demyelinating neuropathy.
43. The method of claim 30 wherein said damaged neural pathway results from a neoplastic lesion.
- 10 44. The method of claim 43 wherein said neoplastic lesion is caused by a neuroblastoma or a glioma.
45. The method of claim 30 wherein said morphogen stimulates cell adhesion molecule production in a cell defining said pathway.
- 15 46. The method of claim 45 wherein said cell adhesion molecule is a nerve cell adhesion molecule.
- 20 47. The method of claim 46 wherein nerve cell adhesion molecule is selected from the group consisting of N-CAM-120, N-CAM-140 and N-CAM-180.
- 25 48. The method of claim 30 or 45 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).

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49. The method of claim 48 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A (fx).  
5
50. The method of claim 49 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).  
10
51. The method of claim 50 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).  
15
52. The method of claim 51 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.  
20
53. The invention of claims 1, 2, 30 or 46 wherein said morphogen comprises a polypeptide chain encoded by a nucleic acid that hybridizes under stringent conditions with the DNA sequence defined by nucleotides 1036-1341 of Seq. Id No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.  
25
54. The invention of claims 1, 2, 26, 30, 45 or 51 wherein said morphogen comprises a dimeric protein species complexed with a peptide comprising a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof.  
30

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55. The invention of claim 54 wherein said dimeric morphogen species is noncovalently complexed with said peptide.
- 5 56. The invention of claims 54 or 55 wherein said dimeric morphogen species is complexed with two said peptides.
- 10 57. The invention of claims 54 or 55 wherein said peptide comprises at least the first 18 amino acids of a sequence defining said pro region.
- 15 58. The invention of claim 57 wherein said peptide comprises the full length form of said pro region.
- 20 59. The invention of claims 54 or 55 wherein said peptide comprises a nucleic acid that hybridizes under stringent conditions with a DNA defined by nucleotides 136-192 of Seq. ID No. 16, or nucleotides 157-211 of Seq. ID No. 20.
- 25 60. The invention of claims 54 or 55 wherein said complex is further stabilized by exposure to a basic amino acid, a detergent or a carrier protein.
- 30 61. A method of maintaining a neural pathway in a mammal comprising:  
administering said mammal an effective amount of an agent capable of stimulating production of an endogenons morphogen in a cell defining said pathway.

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62. A composition for promoting regeneration of a neural pathway at a site of injury in a mammal, comprising:

5 a biocompatible, in vivo bioresorbable carrier suitable for maintaining a protein at a site in vivo, and

10 a morphogen, such that said morphogen, when dispersed in said carrier and provided to said site of injury, is capable of stimulating neural pathway regeneration at said site.

63. The composition of claim 62 wherein said carrier is structurally sufficient to assist direction of axonal growth.

15 64. The composition of claim 63 wherein said carrier comprises a polymeric material.

20 65. The composition of claim 63 wherein said carrier comprises laminin or collagen.

66. A device for repairing a break in a neural pathway, the device comprising:

25 a biocompatible tubular casing comprising an exterior and an interior surface and defining a channel through which a neural process may regenerate,

30 said device having a shape and dimension sufficient to span a break in a neural pathway, and having openings adapted to receive the ends of a severed nerve, and

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5 a morphogen disposed within the channel defined by said tubular casing and accessible to severed nerve ends defining a break in a neural pathway, such that said morphogen stimulates neural pathway regeneration when disposed in said channel and accessible to said nerve ends.

10 67. The device of claim 66 wherein said morphogen is disposed in said channel together with a biocompatible, bioresorbable carrier suitable for maintaining a protein at a site in vivo.

15 68. The device of claim 67 wherein said carrier comprises sufficient structure to assist direction of axonal growth within said channel.

69. The device of claim 67 wherein the outer surface of said casing is substantially impermeable.

20 70. The device of claim 66 wherein said carrier comprises a polymer.

71. The device of claim 67 wherein said carrier comprises laminin or collagen.

25 72. A method for inducing the redifferentiation of transformed cells of neural origin, the method comprising the step of:

30 contacting said transformed cells with a morphogen composition at a concentration and for a time sufficient to induce redifferentiation of said cells to a morphology characteristic of untransformed neuronal cells.



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73. The method of claim 72 wherein said morphology characteristic of untransformed nerve cells includes formation of neurite outgrowths.
- 5 74. The method of claim 72 wherein said morphology characteristic of untransformed nerve cells includes cell aggregation and cell adhesion.
- 10 75. The method of claim 72 wherein said morphogen composition induces nerve cell adhesion molecule production in said cells.
- 15 76. The method of claim 72 wherein said induced nerve cell adhesion molecules include N-CAM-180, N-CAM-140 and N-CAM-120.
77. The method of claim 72 wherein said transformed cells comprise neuroblastoma cells.
- 20 78. A kit for detecting a neuropathy in a mammal or for evaluating the efficacy of a therapy for treating a neuropathy in a mammal, the kit comprising:
- 25     c) means for capturing a cell or body fluid sample obtained from a mammal;
- b) a binding protein that interacts specifically with a morphogen in said sample so as to form a binding protein-morphogen complex;
- c) means for detecting said complex.
- 30 79. The kit of claim 78 which said binding protein has specificity for an epitope defined by part or all of the pro region of a morphogen.

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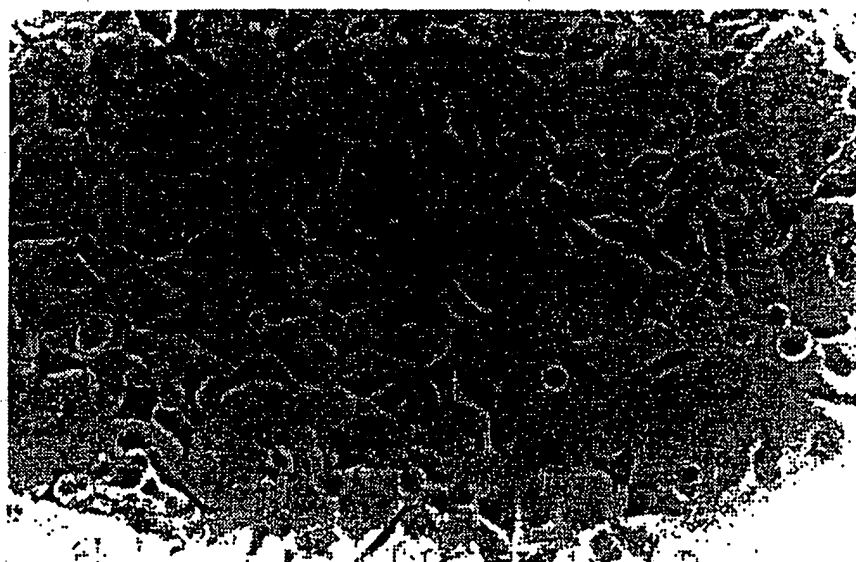
80. A method for detecting a neuropathy in a mammal,  
the method comprising the step of:  
detecting fluctuations in the physiological  
concentration of a morphogen present in the serum  
5 or cerebrospinal fluid of said mammal, said  
fluctuations being indicative of an increase in  
neuronal cell death.
81. A method for detecting a neuropathy in a mammal,  
10 the method comprising the step of:  
detecting fluctuations in the physiological  
concentration of a morphogen antibody titer present  
in the serum or cerebrospinal fluid of said mammal,  
said fluctuations being indicative of an increase  
15 in neuronal cell death.
82. The invention of claims 78, 80 or 81 wherein said  
neuropathy results from a neurodegenerative  
disease, nerve demyelination, myelin dysfunction,  
20 neuronal neoplasias, or nerve trauma.
83. A method of stimulating production of cell adhesion  
molecules in a tissue comprising the step of:  
providing a morphogen to said tissue for a  
25 time and at a concentration sufficient to induce  
production of cell adhesion molecules in cells of  
said tissue.
84. The method of claim 83 wherein said cell adhesion  
30 molecules comprises nerve cell adhesion molecules.
85. The method of claim 84 wherein said cells comprise  
neurons.

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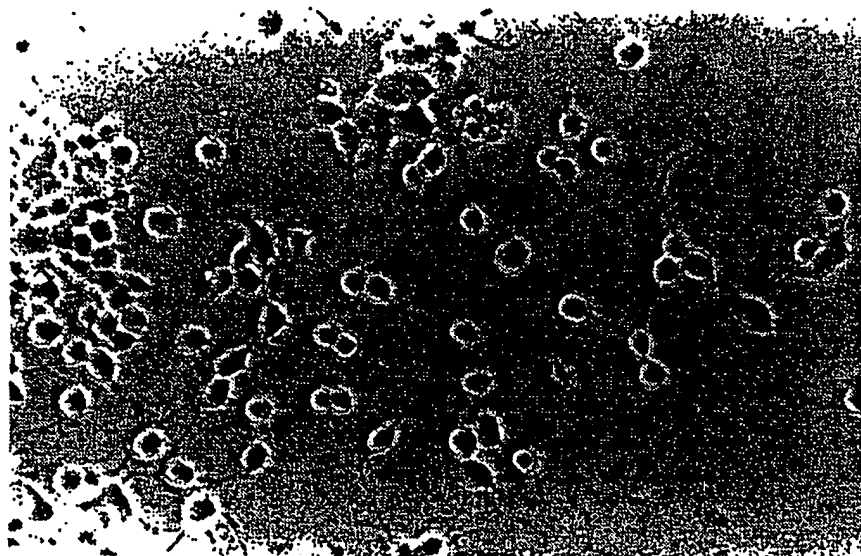
86. The method of claim 78, 80 or 81 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
87. The method of claim 86 wherein said morphogen comprises an amino acid sequence sharing at least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A (fx).
88. The method of claim 87 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
89. The method of claim 88 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
90. The method of claim 89 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
91. The method of claim 78, 80 or 81 wherein said morphogen comprises an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with the sequence defined by nucleotides 1036-1341 of Seq. ID No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.

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92. A composition for enhancing survival of neuronal cells at risk of dying comprising a morphogen in association with a molecule capable of enhancing the transport of said morphogen across the blood-brain barrier.
- 5
93. The invention of claims 62 or 67 wherein said carrier comprises brain tissue derived extracellular matrix.



*Fig. 1A*



*Fig. 1B*

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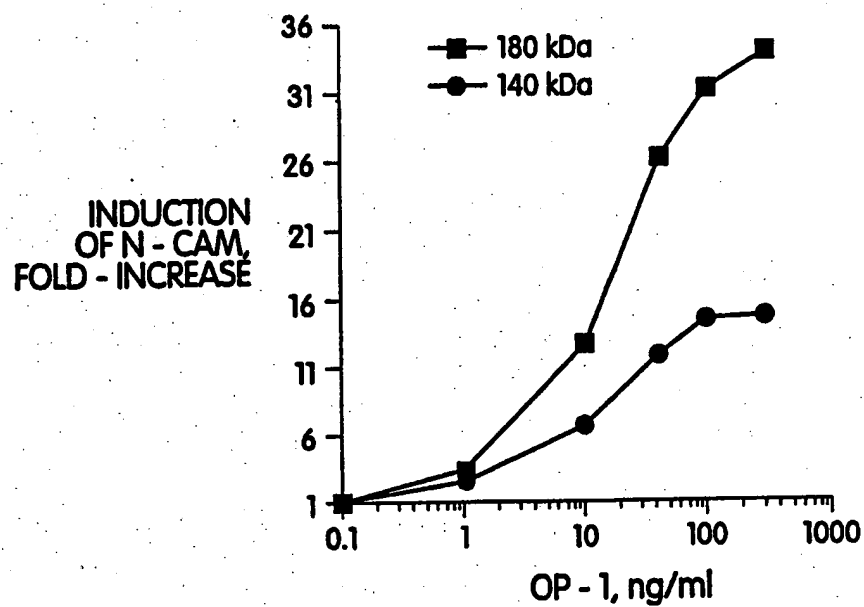


Fig. 2A

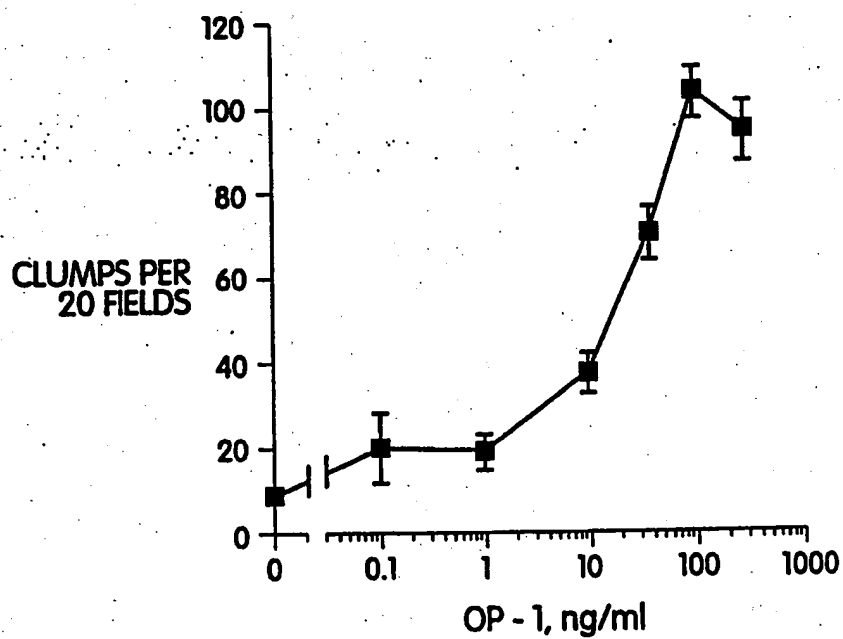
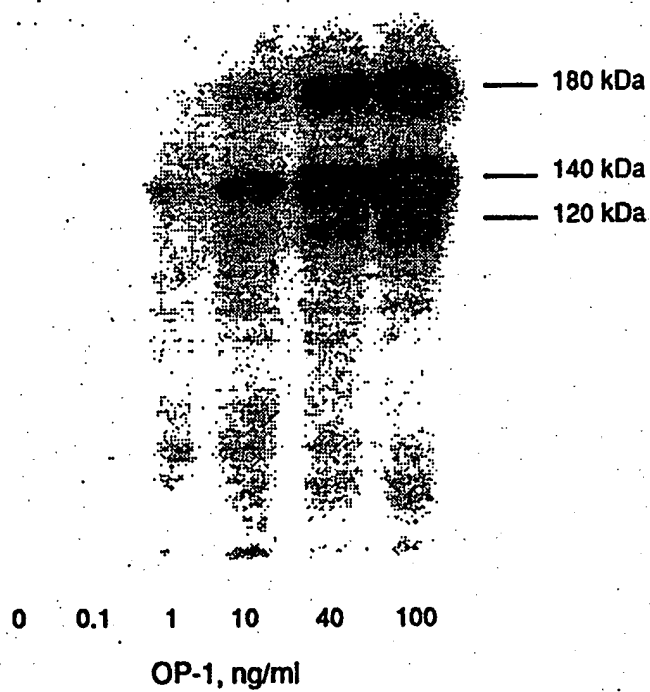
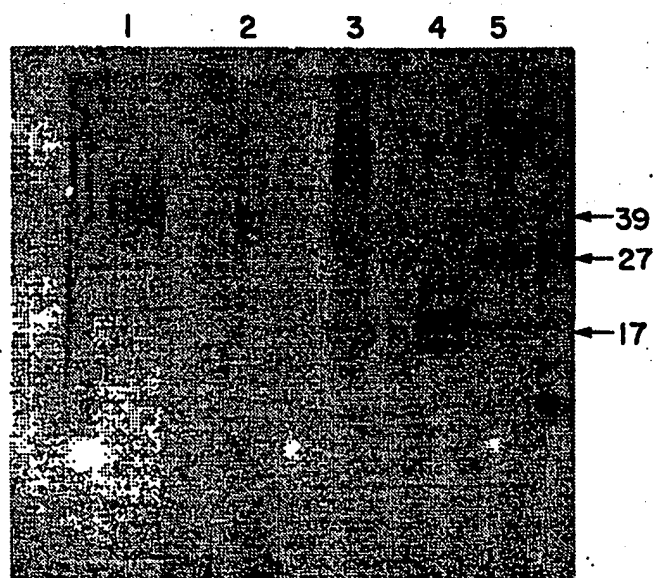


Fig. 3

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**Fig. 2B**



**Fig. 4**

## INTERNATIONAL SEARCH REPORT

Internat Application No  
PCT/US 93/07231A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K37/02 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 00382 (CARNEGIE INSTITUTION OF WASHINGTON) 9 January 1992 see page 9, line 15 - page 15, line 29	1-24,78, 79,82, 86,87
X,P	WO,A,92 15323 (CREATIVE BIOMOLECULES, INC.) 17 September 1992 cited in the application see page 6, line 1 - page 26, line 18	1-93
X,P	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, November 1992, WASHINGTON US pages 10326 - 10330 GEORGE PERIDES ET AL. 'INDUCTION OF THE NEURAL CELL ADHESION MOLECULE AND NEURONAL AGGREGATION BY OSTEOGENIC PROTEIN 1' THE WHOLE ARTICLE	1,20-27, 53

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- \*B\* earlier document but published on or after the international filing date
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*A\* document member of the same patent family

Date of the actual completion of the international search

8 November 1993

Date of mailing of the international search report

07.12.93

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/07231

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BIOLOGICAL ABSTRACTS vol. 91  1991, Philadelphia, PA, US;  abstract no. 106862,  JONES, C. ET AL. 'INVOLVEMENT OF BONE  MORPHOGENETIC PROTEIN-4 (BMP-4) AND VGR-1  IN MORPHOGENESIS AND NEUROGENESIS IN THE  MOUSE'  see abstract  &amp; DEVELOPMENT (CMB)  vol. 111, no. 2, 1991  pages 531 - 542</p> <p>-----</p>	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/07231

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 2,28-52,61,72-77,80,81,83,85 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 93/07231

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9200382	09-01-92	AU-A- 8496491	23-01-92
WO-A-9215323	17-09-92	AU-A- 1754392	06-10-92

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Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at  
res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr,  
Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at  
res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or  
5 Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at  
res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu,  
Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or  
Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa  
at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile,  
10 Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at  
res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 =  
(Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro);  
Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 =  
(Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val,  
15 Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser);  
Xaa at res.100 = (Gly or Ala); and Xaa at res.102 =  
(His or Arg).

Particularly useful sequences for use as  
20 morphogens in this invention include the C-terminal  
domains, e.g., the C-terminal 96-102 amino acid  
residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A,  
CBMP-2B, GDF-1 (see Table II, below, and Seq. ID  
Nos. 5-14), as well as proteins comprising the  
25 C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see  
Seq. ID Nos. 24-28), all of which include at least the  
conserved six or seven cysteine skeleton. In addition,  
biosynthetic constructs designed from the generic  
sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S.  
30 Pat. No. 5,011,691, also are useful. Other sequences  
include the inhibins/activin proteins (see, for  
example, U.S. Pat. Nos. 4,968,590 and 5,011,691).  
Accordingly, other useful proteins are those exhibiting  
morphogenic activity and having amino acid sequences  
35 sharing at least 70% amino acid sequence homology or

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"similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic variants, species variants and other sequence variants (e.g., "muteins" or "mutant proteins"), whether naturally occurring or biosynthetically produced, as well as novel members of this morphogenic family of proteins.

As used herein, "amino acid sequence homology" is understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

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As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et al. (1970) J.Mol. Biol. 48:443-453 and identities calculated by the Align program (DNASTar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

10

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

In still another preferred aspect of the invention, useful morphogens include active proteins comprising polypeptide chains encoded by nucleic acids which hybridize to DNA or RNA sequences encoding the C-terminal sequence defining the conserved cysteine domain, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. Id. Nos. 16 and 20, respectively, of OP1 or OP2 under stringent hybridization conditions. As used herein, stringent hybridization conditions are

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defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

5       The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other  
10   synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including  
15   those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the  
20   specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of  
25   native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

      The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in  
30   procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed  
35   description of the morphogens useful in the methods,

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compositions and devices of this invention is disclosed  
in copending US patent application Serial Nos. 752,764,  
filed August 30, 1991, and 667,274, filed March 11,  
1991, the disclosure of which are incorporated herein  
5 by reference.

Thus, in view of this disclosure, skilled genetic  
engineers can isolate genes from cDNA or genomic  
libraries of various different species which encode  
10 appropriate amino acid sequences, or construct DNAs  
from oligonucleotides, and then can express them in  
various types of host cells, including both procaryotes  
and eucaryotes, to produce large quantities of active  
proteins capable of maintaining neural pathways in a  
15 mammal, including enhancing the survival of neurons at  
risk of dying and stimulating nerve regeneration and  
repair in a variety of mammals, including humans.

The foregoing and other objects, features and  
20 advantages of the present invention will be made more  
apparent from the following detailed description of the  
invention.



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**Brief Description of the Drawings:**

The foregoing and other objects and features of this invention, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

Fig. 1(A and B) are photomicrographs illustrating the ability of morphogen (OP-1) to induce transformed neuroblastoma x glioma cells (1A) to redifferentiate to a morphology characteristic of untransformed neurons (1B);

Fig. 2A is a dose response curve for the induction of the 180 kDa and 140 kDa N-CAM isoforms in morphogen-treated NG108-15 cells;

Fig. 2B is a photomicrograph of a Western blot of whole cell extracts from morphogen-treated NG108-15 cells with an N-CAM-specific antibody; and

Fig. 3 is a plot of the mean number of cell aggregates counted in 20 randomly selected fields as a function of morphogen concentration.

Fig. 4 is a photomicrograph of an immunoblot demonstrating the presence of OP-1 in human serum.

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Detailed Description of the Invention

It now has been discovered that the proteins described herein are effective agents for enhancing the survival of neurons, particularly neurons at risk of dying, and for maintaining neural pathways in a mammal. As described herein, these proteins ("morphogens") are capable of enhancing survival of non-mitotic neurons, stimulating neuronal CAM expression, maintaining the phenotypic expression of differentiated neurons, inducing the redifferentiation of transformed cells of neural origin, and stimulating axonal growth over breaks in neural processes, particularly large gaps in distal axons. The proteins also are capable of providing a neuroprotective effect to alleviate the tissue destructive effects associated with immunologically-related nerve tissue damage. Finally, the proteins may be used as part of a method for monitoring the viability of nerve tissue in a mammal.

20

Provided below are detailed descriptions of suitable morphogens useful in the methods, compositions and devices of this invention, as well as methods for their administration and application, and numerous, nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-stimulating agents described herein as therapeutic agents for maintaining neural pathways in a mammal and enhancing survival of neuronal cells at risk of dying; and 2) provide assays with which to test candidate morphogens and morphogen-stimulating agents for their efficacy.

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## I. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. Details of how the morphogens useful in the method of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in international application US92/01968 (W092/15323), the disclosure of which is hereby incorporated by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat.

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5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

5 Accordingly, the morphogens useful in the methods and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences  
10 described above, where "homology" is as defined herein above.

The morphogens useful in the method of this invention also can be described by any of the 6 generic  
15 sequences described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

20                   1                   5

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human  
25 OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus,  
30 Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A protein (from Drosophila, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method  
35 of Needleman et al. (1970) J. Mol. Biol., 48:443-453,

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calculated using the Align Program (DNASTar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

15	hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
	mOP-1	...	...	...	...	...	...	...	...
	hOP-2	...	Arg	Arg	...	...	...	...	...
	mOP-2	...	Arg	Arg	...	...	...	...	...
20	DPP	...	Arg	Arg	...	Ser	...	...	...
	Vgl	...	...	Lys	Arg	His	...	...	...
	Vgr-1	...	...	...	...	Gly	...	...	...
	CBMP-2A	...	...	Arg	...	Pro	...	...	...
	CBMP-2B	...	Arg	Arg	...	Ser	...	...	...
25	BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
	GDF-1	...	Arg	Ala	Arg	Arg	...	...	...
	60A	...	Gln	Met	Glu	Thr	...	...	...
	BMP5	...	...	...	...	...	...	...	...
	BMP6	...	Arg	...	...	...	...	...	...
30		1				5			
	hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln Asp
	mOP-1	...	...	...	...	...	...	...	...
35	hOP-2	...	...	Gln	...	...	...	...	Leu ...

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	mOP-2	Ser	...	...	...	...	...	...	Leu	...
	DPP	Asp	...	Ser	...	Val	...	...	Asp	...
	Vgl	Glu	...	Lys	...	Val	...	...	...	Asn
	Vgr-1	...	...	Gln	...	Val	...	...	...	...
5	CBMP-2A	Asp	...	Ser	...	Val	...	...	Asn	...
	CBMP-2B	Asp	...	Ser	...	Val	...	...	Asn	...
	BMP3	Asp	...	Ala	...	Ile	...	...	Ser	Glu
	GDF-1	...	...	...	Glu	Val	...	...	His	Arg
	60A	Asp	...	Lys	...	...	...	...	His	...
10	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	Gln	...	...	...	...	...	...
			10					15		
	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
15	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	Val	...	...	...	Gln	...	...	Ser
	mOP-2	...	Val	...	...	...	Gln	...	...	Ser
	DPP	...	...	Val	...	...	Leu	...	...	Asp
	Vgl	...	Val	...	...	...	Gln	...	...	Met
20	Vgr-1	...	...	...	...	...	Lys	...	...	...
	CBMP-2A	...	...	Val	...	...	Pro	...	...	His
	CBMP-2B	...	...	Val	...	...	Pro	...	...	Gln
	BMP3	...	...	...	Ser	...	Lys	Ser	Phe	Asp
	GDF-1	...	Val	...	...	...	Arg	...	Phe	Leu
25	60A	...	...	...	...	...	...	...	...	Gly
	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	...	...	...	Lys	...	...	...
				20					25	
30	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	...	...	...	...	...	...	...	Ser
	mOP-2	...	...	...	...	...	...	...	...	...
35	DPP	...	...	...	...	His	...	Lys	...	Pro

[illegible]

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	CBMP-2B	...	...	...	...	...	...	...	...	...
	BMP3	Ser	...	...	...	Thr	Ile	...	Ser	Ile
	GDF-1	Leu	...	...	...	Val	Leu	Arg	Ala	...
	60A	...	...	...	...	...	...	...	...	...
5	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	...	...	...	...	...	...	...
		45					50			
10	hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
	mOP-1	...	...	...	...	...	...	Asp	...	...
	hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
	mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
	DPP	...	Asn	Asn	Asn	...	...	Gly	Lys	...
15	Vgl	...	...	Ser	...	Glu	...	...	Asp	Ile
	Vgr-1	...	...	Val	Met	...	...	...	Tyr	...
	CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
	CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
	BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
20	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
	60A	...	...	Leu	Leu	Glu	...	Lys	Lys	...
	BMP5	...	...	Leu	Met	Phe	...	Asp	His	...
	BMP6	...	...	Leu	Met	...	...	...	Tyr	...
			55					60		
25										
	hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	...	Ala	...	...	...	...	...	Lys
30	mOP-2	...	...	Ala	...	...	...	...	...	Lys
	DPP	...	...	Ala	...	...	Val	...	...	...
	Vgl	...	Leu	...	...	...	Val	...	...	Lys
	Vgr-1	...	...	...	...	...	...	...	...	Lys
	CBMP-2A	...	...	Ala	...	...	Val	...	...	Glu
35	CBMP-2B	...	...	Ala	...	...	Val	...	...	Glu



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5	BMP3	...	Glu	...	...	...	Val	...	Glu	Lys
	GDF-1	Asp	Leu	...	...	...	Val	...	Ala	Arg
	60A	...	...	...	...	...	...	...	...	Arg
	BMP5	...	...	...	...	...	...	...	...	Lys
	BMP6	...	...	...	...	...	...	...	...	Lys
				65					70	
10	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
	mOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
	Vgl	Met	Ser	Pro	...	...	Met	...	Phe	Tyr
	Vgr-1	Val	...	...	...	...	...	...	...	...
15	DPP	...	Asp	Ser	Val	Ala	Met	...	...	Leu
	CBMP-2A	...	Ser	...	...	...	Met	...	...	Leu
	CBMP-2B	...	Ser	...	...	...	Met	...	...	Leu
	BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
	GDF-1	...	Ser	Pro	...	...	...	...	Phe	...
	60A	...	Gly	...	Leu	Pro	...	...	...	His
20	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	...	...	...	...	...	...	...
					75					80
25	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
	mOP-1	...	...	...	...	...	...	...	...	...
	hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
	mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
	DPP	Asn	...	Gln	...	Thr	...	Val	...	...
	Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
30	Vgr-1	...	...	Asn	...	...	...	...	...	...
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
	BMP3	...	Glu	Asn	Lys	...	...	Val	...	...

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	GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg
	60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
	BMP5	...	...	...	...	...	...	...	...	...
	BMP6	...	...	Asn	...	...	...	...	...	...
5						85				
	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	
	mOP-1	...	...	...	...	...	...	...	...	
10	hOP-2	...	His	...	...	...	...	...	Lys	
	mOP-2	...	His	...	...	...	...	...	Lys	
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val	
	Vgl	His	...	Glu	...	...	Ala	...	Asp	
	Vgr-1	...	...	...	...	...	...	...	...	
15	CBMP-2A	Asn	...	Gln	Asp	...	...	...	Glu	
	CBMP-2B	Asn	...	Gln	Glu	...	...	...	Glu	
	BMP3	Val	...	Pro	...	...	Thr	...	Glu	
	GDF-1	Gln	...	Glu	Asp	...	...	...	Asp	
	60A	...	...	...	...	...	Ile	...	Lys	
20	BMP5	...	...	...	...	...	...	...	...	
	BMP6	...	...	...	Trp	...	...	...	...	
		90					95			
25	hOP-1	Ala	Cys	Gly	Cys	His				
	mOP-1	...	...	...	...	...				
	hOP-2	...	...	...	...	...				
	mOP-2	...	...	...	...	...				
	DPP	Gly	...	...	...	Arg				
30	Vgl	Glu	...	...	...	Arg				
	Vgr-1	...	...	...	...	...				
	CBMP-2A	Gly	...	...	...	Arg				
	CBMP-2B	Gly	...	...	...	Arg				
	BMP3	Ser	...	Ala	...	Arg				
35	GDF-1	Glu	...	...	...	Arg				